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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 48/00

A1

(11) International Publication Number: WO 99/03505

(43) International Publication Date: 28 January 1999 (28.01.99)

(21) International Application Number: PCT/GB98/02150

(22) International Filing Date: 17 July 1998 (17.07.98)

(30) Priority Data:
9715085.8 18 July 1997 (18.07.97) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GENE THERAPY VEHICLE COMPRISING DERMAL SHEATH TISSUE

(57) Abstract

The invention herein described relates to a gene therapy vehicle, comprising dermal sheath tissue and/or cells derived from portions of hair follicles which show pluripotentiality, and which has use in the delivery of therapeutic agents to selected tissues and advantageously has the potential to repair/replace damaged tissue.

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GENE THERAPY VEHICLE COMPRISING DERMAL SHEATH TISSUE

The invention relates to the use of dermal sheath tissue and/or cells derived therefrom and/or portions of hair follicles containing these and other cell populations for use particularly, but not exclusively in gene therapy/vaccine development.

Human gene therapy vectors constructed to date are typically derived from viruses (1). The rationale being that such vectors can easily penetrate cells by virtue of naturally infecting human cells and so can incorporate fragments of foreign DNA into a target cell population. The most widely investigated viruses are of the adenovirus, retrovirus, parvovirus and herpesvirus families. With the exception of retroviruses, all have been derived from viruses originally isolated from humans. In nearly every case the vectors used in both *ex* and *in vivo* work have been derived from virus mutants originally created to study gene function, rather than to act as gene delivery systems.

Although adenoviruses have proved to be popular because of ease of growth of stocks to high titre, they have many associated problems. For example it is known that viruses which are replication incompetent in cell culture have caused tissue damage and respiratory disease in patients treated with such vectors (2).

Herpesvirus vector development to date has concentrated on derivatives of the common human pathogen herpes simplex virus (HSV). The advantage of using this virus is that it is the most intensively studied of all the herpesviruses. The sequence of the virus genome has been determined, there is a wide range of well characterised virus mutants available and transcriptional control processes are

well understood. However the disadvantage with this virus is that the mutant virus is difficult to produce as high titre stocks and in some cases has an unacceptable reversion frequency. Additionally, it is a likely problem with HSV vectors that there is an innate immune response present in the majority of the population; it is predicted that HSV vectors will suffer the same problem as those derived from human adenoviruses, when delivered to an immunologically competent site.

Additionally, and more recently, naturally occurring specific cell populations have been investigated as gene therapy delivery systems however such systems have to date only employed self-derived cells and consequently are limited to the disease state of the individual from which the cells are derived. Such systems suffer from immunological repercussions and have not produced particularly encouraging results nor do they offer the possibility of inter and/or intra species therapies.

Thus a vehicle capable of efficient and immunopriviledged gene delivery to human cells would have a wide range of uses in human gene therapy, for example delivery of a correct copy of human tumour suppressor genes to tumours of a variety of different organs and/or as a vaccine delivery vehicle to induce specific immunity.

Skin is a highly complex organ covering the external surface of the body and merging, at various body openings, with the mucous membranes of the alimentary and other canals. It has multiple functions such as preventing water loss from the body, but predominantly acts as a protective barrier against the action of physical, chemical and bacterial agents on deeper tissues. Skin is elastic and except for a few areas such as the palms, soles and ears it is loosely attached to underlying tissue. It varies in thickness from 0.5mm (0.02 inches)

on the eyelids to 4mm (0.17 inches) or more on the palms and soles.

Skin is composed of two layers (please refer to Figure 1 which illustrates an anatomical cross-sectional view through a slice of skin), the outer layer, which is comparatively thin (0.1 mm) is called the epidermis, or cuticle, it is several cells thick and has an external, horny layer of dead cells that are constantly shed from the surface and replaced from below by a basal layer of cells, called the stratum germinativum. The epidermis is composed predominantly of keratinocytes which make up over 95% of the cell population, the rest include dendritic cells such as Langerhans cells and melanocytes. It is essentially cellular and non-vascular, there being relatively little extracellular matrix except for the layer of collagen and other proteins beneath the basal layer of keratinocytes. Keratinocytes of the basal layer are constantly dividing, and daughter cells subsequently move outwards, where they undergo a period of differentiation and are eventually sloughed off from the surface. The inner layer of the skin is called the dermis and is composed of a network of collagenous extracellular material, elastic fibres, blood vessels and nerves. Contained within it are hair follicles with associated sebaceous glands (collectively known as the pilosebaceous unit) and sweat glands. The interface between the epidermis and dermis is extremely irregular and consists of a succession of interdigitations, or finger like projections. Beneath the basal epidermal cells along this interface the specialised extracellular matrix is organised into a distinct structure called the basement membrane.

The mammalian hair fibre is the product of a small but complex, cylindrical arrangement of tissues known as the hair follicle. Follicles lie angularly underneath the skin's surface, their distal most epidermis being in direct continuation with that of the skin at the point where they open externally. Although small, the follicle comprises a highly organised system of

recognisably different layers arranged in concentric series. Active hair follicles extend down through the dermis, the hypodermis (a loose layer of connective tissue), and the fat or adipose layer.

At the base of any active follicle lies the hair bulb, which consists of a body of dermal cells, known as the dermal papilla, contained in an inverted cup of epidermal cells known as the epidermal matrix (please refer to Figure 1). Irrespective of follicle type, the hair fibre, together with several supportive epidermal layers, is produced by germinative epidermal cells at the very base of this epidermal matrix. The lowermost dermal sheath is contiguous with the papilla basal stalk, from where it curves externally around all of the epidermal layers of hair matrix as a thin covering of tissue, and then continues as a tube or sleeve for the length of the follicle. The dermal sheath is otherwise known as the connective tissue sheath.

Developing skin appendages such as feather and hair follicles rely on interaction between the skin's two layers, the epidermis and the dermis. In embryonic development, a sequential exchange of information between these layers underpins a complex series of morphogenetic processes culminating in the formation of adult follicle structures. However, following maturity, and in contrast to general skin dermal and epidermal cells, certain hair follicle cell populations retain embryonic-type inductive, interactive and biosynthetic behaviours. These properties are likely to derive from the very dynamic nature of the cyclically productive follicle, in which repeated tissue remodelling necessitates a high level of dermal-epidermal interactive communication, as is vital for embryonic development and, as would be desirable in any form of tissue reconstruction.

Hair fibre is produced at the base of an active follicle at a very rapid rate

(0.4mm per day in the human scalp follicles and up to 1.5mm per day in the rat vibrissa or whiskers), which means that cell proliferation in the follicle epidermis ranks amongst the fastest in adult tissues (3).

The most dynamic region of the hair follicle is the deeply embedded end bulb, where local dermal-epidermal interactions drive active fibre growth. This same region is also central to the developmental changes and tissue remodelling involved in the hair fibre's or appendages precise alternation between growth and regression phases. As a key player in the activities, the dermal papilla appears to orchestrate the complex program of differentiation that characterises hair fibre formation from the primative germinative epidermal cell source (4-7). The lowermost dermal sheath initiates below the papilla basal stalk, from where it curves outwards and upwards to externally enclose all of the layers of the epidermal hair matrix as a thin cup of tissue. (Please refer to Figure 1). The dermal sheath continues as a tubular arrangement for the length of the follicle, as does the epidermal outer root sheath which lies immediately internal to it in between the two layers is a specialised basement membrane termed the glassy membrane. The outer root sheath constitutes little more than an epidermal monolayer in the lower follicle, but becomes increasingly thickened more superficially.

Whilst the individual anatomical components and cell sub-populations of skin are well established their intra/inter biochemical interactions and control mechanism remains largely a matter for speculation and intense research.

The most important of all cells types are those at the source of every biological system ie stem cells, since they vitally sustain and replenish the more differentiated descendent population and as they become specialised develop a characteristic function. Yet these are the cells which are least understood in

terms of their distribution, behaviour and the factors by which they may be defined. The ability to provide significant numbers of pure, unstimulated, undifferentiated, primitive stem cells from an adult organ would be likely to have a broad impact on our fundamental understanding of cell biology, and would yield positive and promising approaches to future therapeutic advances.

Serendipitously, we have studied hair follicles and identified a specific cell population with immunoprivilege and stem specific cell potential that can be used most advantageously as a cellular delivery system in gene therapy.

We have found that the implantation of male follicle-derived dermal sheath cells into a female recipient does not lead to the typical immune response and subsequent rejection that one would expect. The same observation held true even after a subsequent set of implantations with the same human host and donor, when second set rejection would have been predicted. Such results show that dermal sheath cells have some form of privileged immune status. A number of our tissue interaction/induction studies have also clearly demonstrated that cells derived from different species appendages are very capable of interacting with each other, and communicating at the appropriate levels to allow complex morphogenesis. This being the case, dermal sheath tissue and/or cells derived therefrom represent a cell population of major consequence in gene therapy as vehicles for both inter and intra species therapy delivery. Additionally the ability of dermal sheath tissue and/or cells derived therefrom to differentiate into a variety of different phenotypes makes their contribution to gene therapy even more significant, in that using such cells as vehicles means that not only would they be tolerated in multiple and different tissue/cell sites, but that they would also be more effective and penetrating by differentiating into multiple tissue types depending on the site of delivery. Further natural attributes that pre-dispose follicle cells as candidates for the

application of gene therapy include: their similarity to wound myofibroblasts; their exhibition of stem cell-type qualities including those characterising primative muscle lineages, indeed follicle-derived muscle stem cells are especially suited to gene therapy applications because of their ability to fuse with other cells. Furthermore since dermal sheath cells have many of the properties of smooth muscle cells they have an additional potential in vascular related therapy by incorporation into blood vessels as the smooth muscle component; production of a unique embryonic-type extracellular matrix and, the fact that they exhibit impressive regenerative and inductive abilities.

It is therefore an object of the invention to provide a new gene therapy system that employs follicle derived cells/tissues and/or their attributes.

It is a yet further object of the invention to provide an inter or intra species gene therapy that employs follicle derived cells/tissues and/or their attributes.

It is a yet further object of the invention to provide a gene therapy vehicle having multi-potential incorporation and differentiation properties.

According to a first aspect of the invention there is provided dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles for use in a gene therapy.

According to a further aspect of the invention there is provided a gene therapy vehicle for delivering at least one selected gene, or functional fragment thereof, to a target site comprising dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles.

Reference herein to a functional fragment thereof is intended to include a part

of a gene that provides for the expression of the corresponding protein or an active or effective part thereof.

Reference herein to cells typically closely associated with hair follicles is intended to include cells that are functionally and/or locationally associated with and/or within hair follicles.

In a preferred embodiment of the invention said dermal sheath tissue and/or said cells derived therefrom and/or said cells typically closely associated with hair follicles is/are derived from a selected portion of a follicle ideally the lower third thereof and even more ideally are derived from a segment or ring of a combination of follicle tissue/cells.

In a yet further preferred embodiment of the invention said gene therapy vehicle is suitably engineered, ideally using recombinant techniques, so as to include at least one insertion site into which at least one selected gene can be placed. Those skilled in the art will appreciate that the provision of this insertion site allows the gene therapy vehicle to carry a selected gene to a desired location. More preferably still said selected gene is functionally inserted into said gene therapy vehicle so that the expression of said gene results in the provision of the corresponding protein product. It would be understood by those skilled in the art that the nature of the gene to be inserted will be selected having regard to the purpose of the gene therapy vehicle and thus the nature of the condition to be cured, treated or alleviated. In addition, said gene therapy vehicle may be provided with multiple insertion sites with a view to carrying multiple genes and so providing for the delivery of multiple proteins, either of a similar or different nature. In each instance, said selected gene for insertion is arranged so as to be inserted in-frame with the genome of the gene therapy vehicle so as to provide for correct expression of same.

In a yet further preferred embodiment of the invention said gene therapy vehicle comprises at least one selected gene or functional fragment thereof which is operationally attached to a regulatable or inducible or a constitutive promoter.

In a yet further aspect of the invention there is provided a vector for transforming or transfecting the gene therapy vehicle of the invention wherein said vector is provided with at least one insertion site into which at least one selected gene can be placed and also other expression control elements for ensuring that once the vector infects or penetrates said tissue and/or cells derived therefrom expression of the said selected gene can take place.

In a yet further preferred embodiment of the invention there is provided a therapeutic composition comprising a suitable carrier for the gene therapy vehicle in accordance with the invention, ideally said carrier can be formulated to have anti-bacterial properties and/or anti-septic properties and more ideally further include growth promoting additives and/or local anaesthetics. Ideally said therapeutic composition may be adapted to be applied topically in the form of dermal sheath cells suspended in a suitable carrier solution/gel/cream/emollient; alternatively said composition may be adapted to be administered by injection and so comprise a carrier solution; alternatively still, said carrier may be incorporated and/or embedded therein and/or associated therewith and/or attached thereto a plaster or bandage or the like.

According to a further aspect of the invention there is provided a potential gene therapy vehicle for use in delivering a selected gene, or functional fragment thereof, to a given site wherein said gene therapy vehicle comprises dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles which cells and/or tissue that have been suitably

adapted to accommodate heterologous genetic material and which, *in vivo*, have the capacity to selectively differentiate to provide at least one differentiated tissue type.

It will be apparent to those skilled in the art that, given the pluripotentiality of these cells, that the site of implantation will, to some extent, determine the differentiated pathway along which these cells will develop. Thus, the site of implantation will determine the nature of the phenotype of these cells and therefore one is provided with a gene therapy vehicle that not only is able to deliver at least one selected gene but which also has the added advantage of being able to provide differentiated tissue. This feature is particularly important where an individual may have suffered tissue damage, for example, following wounding of any type or following ischemia or vascular damage, or even removal of at least part of an organ or tissue.

It would therefore be seen that, advantageously, the gene therapy vehicle of the invention may be suitably cultured for the purpose of implantation and/or suitably impregnated onto wound healing materials such as bandages or seeded into biomaterials or coated onto replacement blood vessels or the like.

In the instance where the gene therapy vehicle is to be used in relation to wound healing said dermal sheath tissue and/or said cells derived therefrom and/or cells typically closely associated with hair follicles are provided or combined with at least one other appropriate cell type from a hair follicle. This combination is favoured because our experiments have shown that dermal papilla tissue, or cells derived therefrom may assist in the closure of wound and in the reduction of scar tissue.

In a yet further preferred embodiment of the invention there is provided a

wound healing system comprising a suitable matrix material having incorporated and/or embedded therein and/or associated therewith and/or attached thereto a gene therapy vehicle in accordance with the invention, ideally said matrix material comprises native collagen or collagenous gels or lattices constructed from reconstituted collagen or highly complex mixtures of reconstructed collagen and a multitude of extracellular matrix products or any other suitable matrix material known to those skilled in the art, the selection of which is not intended to limit the scope of the invention.

In a yet further preferred embodiment of the invention there is provided a surgical dressing comprising a web material and a suitable matrix material, at least one of which materials has incorporated and/or embedded therein and/or associated therewith and/or attached thereto a gene therapy vehicle in accordance with the invention, ideally said surgical dressing is conventional, the selection of which is not intended to limit the scope of the invention.

According to a yet further aspect of the invention there is provided a wound healing system as hereinbefore described for use in treatment of acute and/or chronic and/or minor and/or severe wound healing; and/or cartilage repair and/or bone repair and/or muscle repair and/or connective tissue repair and/or blood vessel repair.

In summary, we believe the dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles have an important part to play in gene therapy because this tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles:-

i) exhibit immunoprivilege,

ii) exhibit the capacity to incorporate themselves within disturbed tissue sites and fuse directly with host cells,

iii) exhibit multipotentiality in terms of the differentiated cell lineages they can follow,

iv) exhibit interactive flexibility both in terms of merging within different body sites and also surviving and interacting within different species,

v) exhibit longevity and general durability, e.g. can be stored long term at low temperatures and still retain the aforementioned properties,

vi) advantageously are of adult origin and since most gene therapies will be aimed at adults the gene therapy of the invention provides the benefits of embryonic - type properties without the potential risk of utilising genuine embryo derived cells,

vii) represent a relatively rich deposit of stem cells,

viii) promote healing thereby reducing scarring and delay fibro-fatty deposit accumulation,

ix) have the ability to pass through the basement membrane, by virtue of the production of large amounts of metalloproteinases, as seen in lower follicle regeneration when sheath cells move through basement membranes on the way to becoming papilla cells (8, 9), thus these cells have the potential to reach parts of the body remote from site of delivery.

Thus the invention presents a gene therapy delivery system that can be reliably

manufactured and then stored for future use. Additionally, this tissue and/or cells derived therefrom can exist for a long time in culture under extreme stress, and accordingly presents a gene therapy delivery system that is robust in nature, another favourable advantage in terms of storage, and subsequent application.

An embodiment of the invention will now be described by way of example only with reference to the following Figures wherein:-

Figure 1 represents a diagrammatic illustration of an anatomical cross-sectional view through a slide of skin:

A external hair fibre;

B interfollicular epidermis of skin;

C general interfollicular dermis;

D sebaceous gland;

E epidermal outer root sheath (shown in solid black);

F dermal sheath (broken line 1);

G epidermal inner root sheath (thin layer around fibre);

H dermal papilla (central pear shape);

I germinative epidermal cells (form a tight collar around papillar base).

Figure 2 represents a diagrammatic representation of procedures;

A. male scalp

A1 heals and upper follicle portions regenerate to restore pre-biopsy state

B. punch biopsy taken

B1. Punch biopsy replaced on scalp

C. end bulbs amputated

D. end bulbs dissected

E. to provide various tissue components

F. isolated papilla

G. isolated sheath

H. pooled dermal papillae

I. pooled dermal sheath

J. sheath and papillae transplanted into female forearm skin

K. female arm where male tissue has induced hair follicle neogenesis!

Figure 3 represents pictorial evidence of isolated dermal papilla (P) and sheath (S) tissue microdissected from male scalp hair follicle end bulbs: as shown in Figure 2e, marked by a star (*).

Figure 4 represents pictorial evidence of two hair fibres which have been produced in the immediate vicinity of the male dermal sheath-implanted female skin wound protected by a small silicone rubber collar.

Figure 5 represents pictorial evidence of Figure 4 after the silicone collar (and plaster attachment) has been removed.

Figure 6 represents pictorial evidence of a histological section through an end bulb region of an induced follicle, revealing an Alcian blue-positive stained papilla (P).

Figure 7 represents pictorial evidence of a lower portion of an induced follicle which can be seen to stain positively following in situ hybridisation with a Y-chromosome-specific DNA probe, realised via digoxygenin label.

Figure 8 represents pictorial evidence of a tissue section acting as negative control for Figure 7 and represents female skin that is not stained at all by the digoxygenin-linked Y-chromosome probe.

Figure 9 represents pictorial evidence of a lower portion of an induced follicle stained positively following in situ hybridisation with a Y-chromosome-specific DNA probe, realised via a green fluorophore marker.

Figure 10 represents pictorial evidence of a tissue section acting as a positive control for Figure 9.

Figure 11 represents pictorial evidence of a high power magnification view of the side of a long term [24 days] graft.

Figure 12 represents pictorial evidence of dermal sheath cell capability to differentiate into different mesenchymal cells.

(A) Long term cultured (over a year) human dermal sheath cells.

(B) Dermal sheath cells appearing to fuse in myoblast (muscle-like) fashion.

(C) Myotube-like structures in dermal sheath cell cultures.

(D) Adipocyte (fat producing) cells.

(E) Chondrocyte (cartilage-type) cells.

(F) Mineral producing bone precursor cells - Von Kossa stained.

(G) Dermal sheath cells labelled immunohistochemically for alpha- smooth muscle actin.

(H) Human dermal sheath cells positively stained for smooth muscle myosin.

(I) Dermal sheath cells labelled positively for desmin.

Figure 13 represents pictorial evidence of skin at the margin of a wound and in which dermal sheath cells have surrounded an isolated follicle in the undamaged tissue away from the main group of labelled cells remote in undamaged tissue.

Figure 14 represents a schematic representation of an e-GFP construct for transforming dermal sheath cells.

Figure 15 represents a schematic representation of a method for inserting the e-GFP gene into a vector.

Figure 16 represents pictorial evidence of transfected dermal sheath cells with a construct containing enhanced green fluorescent protein e-GFP and a constitutive promoter.

Experimental Approach

Tissue isolation

A small patch of male scalp skin (about 1.5 cm^2) was coarsely shaven, leaving some fibre still exposed to allow for subsequent plucking. The area was wiped with an antiseptic solution and injected locally with lignocaine plus adrenaline anaesthesia, before taking a 6 mm diameter punch biopsy at an angle appropriate to follicle orientation. The most proximal tips (under 1/5th of length) of the exposed follicles were amputated under a dissecting microscope (Zeiss) from the inverted biopsy, and transferred to individual drops of minimal essential medium (Gibco) at 4^0C. After plucking the hair fibres from the transected follicles, the biopsy was returned to its original scalp skin site and left to heal. This initial procedure lasted about 20-25 mins. Refer to Figure 2 (a, a1, b, b1 and c) which represents a diagrammatic representation of procedures.

The outermost end bulb dermal layers were inverted to allow the epidermal matrix (including undifferentiated tissue) to be scraped away and discarded (Fig. 2d). Dermal papillae, isolated by basal stalk severance (Fig. 2e), were pooled in fresh medium (Fig. 2h). The thin external covering of connective tissue was then teased from the pieces of sheath dermis before they were similarly pooled in fresh medium. (Fig. 2g and i). Figure 3 represents pictorial evidence of isolated dermal papilla (P) and sheath (S) tissue microdissected from male scalp hair follicle end bulbs as shown in Figure 2e marked by a star (*).

Implantati ns

These operations were so minimally invasive as to be practically imperceivable, hence, no form of local anaesthetic pretreatment was deemed necessary. This also avoided the possibility that the anaesthetic might adversely affect the tiny quantities of vulnerable dermis that were to be implanted.

A small, shallow wound was made in the inner forearm of the female recipient with the point of a scalpel blade, and; widened slightly using the tips of very fine (No.5) watchmakers forceps (Fig. 2j). In the few instances when a tiny amount of blood or fluid was exuded, it was absorbed using tiny sterile cotton wool balls. Two sets of operations were performed.

In the first, dermal sheath tissue from twelve follicles were implanted into two wound sites (six in each), approximately 10 hours after the end bulbs had been removed from the biopsy. The second, involved the implantation of 11 pieces of dermal sheath into one wound site, 9 dermal papillae into a second, and 2 papillae (which stuck to the forceps and had to be re-implanted separately) into a third, about 20 hours after biopsy. In all cases, the material was collected in as little fluid as possible and then transferred directly to the wound site, so that it could be rapidly inserted into the skin on the end of the forceps. The wounds were initially left untreated and uncovered. When hair fibres were seen emerging from the implanted sites (3-4 weeks later), small silicone rings with rims were placed over them and secured using surgical tape - as a cautionary measure to protect against abrasion, please refer to Figure 4 which represents pictorial evidence of two hair fibres which have been produced in the immediate vicinity of the male dermal sheath-implanted female skin wound protected by a small silicone rubber collar and Figure 5 which represents pictorial evidence of Figure 4 after the silicon collar (and plaster attachment) have been removed.

The first set of two wound sites were biopsied together as a single piece of elliptical skin, 77 days after sheath tissue implantation, and were fixed immediately in freshly prepared 4% paraformaldehyde at pH 7.3. The second set of wounds (made 3 months after the first) were treated similarly - being removed 42 days post-operatively as two small (4 mm) punch biopsies (more precisely located by their positioning next to moles). Detailed external observations and photographic recordings of the male donor scalp, and recipient female arm skin sites, were made at regular intervals.

Fluorophore-labelled Y-chromosome probe [Imagenetics]

The spectrum green fluorophore-labelled enumerator probe (Imagenetics), consisted of chromosome-specific sequences from highly repeated human satellite DNAs. The target DNA in the tissue sections was denatured in 70% formamide / 2x SSC at 70^{0}C for 10 mins. Meanwhile, the probe mixture was prepared to contain: 7µl SpectrumCEP hybridisation buffer (dextran sulphate, formamide, SSC, pH 7.0), 1µl SpectrumCEP probe (fluorophore-labelled enumerator probe and blocking DNA in Tris-EDTA buffer) and 2µl of 5x blocking solution (x number of slides), which were centrifuged (1-3 secs), heated for 5 mins in a 75^{0}C water bath and then placed on ice. The denatured slides were washed in 70%, 85% and 100% ethanol (1 min in each) and then air dried. Each slide, heated to 45^{0}C, received 10 µl of probe mix and then a silanised coverslip which was sealed at the edges prior to the slides incubation in a humid box at 42^{0}C for 18 hours. Following hybridisation and coverslip removal, the slides were washed for: 3x 10 mins in 50% formamide / 2x SSC; 10 mins in 2x SSC, and 5 mins in 2x SSC / 0.1% NP-40, all containing Denhardts solution, 50 µg/ml sonicated salmon sperm DNA,1% milk powder

and 0.1% Tween-20 and all at 45°C. The slides were allowed to air dry in the dark, and then 10 µl of propidium iodide counterstain (Imagenetics) and a coverslip, added to each.

Digoxigenin-labelled Y-chromosome probe [Boehringer Mannheim]

Each slide received 20 µl of the hybridisation mixture, consisting of: 10 µl formamide [50% of final volume]; 5 µl 4X hybridisation solution; 2.5 µl probe [50 ng]; 2.5µl 8X blocking solutions. The mixture was covered by a silanised glass coverslip, sealed and then denatured for 5->10 mins at 72°C on a pre-warmed glass plate in the oven, before incubation in a moist chamber at 37°C overnight. The slides were washed for 3X 5 mins in 2X SSC, prior to 30 mins in 50 ml TBS containing 1X blocking solution (as above) and 1% Boehringer kit blocker reagent - both also at 37°C. To promote detection, the slides were transferred to 50 ml TBS and 50 µl anti-digoxigenin alkaline phosphatase conjugate [200 µg/ml] containing 1% kit blocker reagent for 30 mins at 37°C, and then they were washed for 3X 10 mins in 0.2% Tween 20 in TBS at room temperature. Immediately before use, 4.5 µl of NBT, 3.5 µl of X-phosphate and 0.24 mg of levamisole (Sigma) was added to 1ml of Tris / NaCl / $MgCl_2$ buffer. Appropriate volumes for the number and size of the sections were added and the slides incubated at room temperature in a humidified box covered in foil until the dark blue/purple colour developed. To stop the reaction, the slides were rinsed for 5 mins at room temperature in 10 mM Tris-Cl / 1 mM Na2 EDTA, pH 8.0.

Sections to be counter stained with propidium iodide were incubated for 5 mins at room temperature in the dark in 50 ml TBS + 5 ul propidium iodide [1mg / ml], or a similar concentration of acriflavine yellow, washed for 2-3 mins under

running water, and then allowed to air dry in the dark. Finally, the sections were mounted in 20 µl of anti-fading solution under a glass coverslip, which was sealed at the edges with nail varnish.

Transfection of Dermal Sheath Cells Cultured From Rat Vibrissa Follicles

Rat dermal sheath cells cultured from vibrissa follicles were transfected using lipofectamine, according to the following procedure. 1-3 X 10^5 cells were seeded per well in 2ml of the appropriate complete growth media and plated into a six-well or 35-mm tissue culture plate. The cells were then incubated at 37^0C in a CO_2 incubator until the cells were 50-80% confluent. This procedure usually lasted 18 to 24 hours. The following solutions were prepared for each transfection, solution A contained dilute 1-2 µg of DNA into 100 µg serum free medium, typically OPTI-MEM® reduced serum medium (GIBCO BRL CAT.NO.31985). Solution B contained for each transfection, dilute 2-25 µl of lipofectamine reagent into 100 µl of serum free medium. Subsequently the solutions A and B were mixed gently and incubated at room temperature for 15 to 45 minutes so as to allow the DNA liposome complexes to form. Further serum-free medium was added to each tube containing the complexes, and cells were incubated with complexes for 2 to 24 hours at 37^0C in a CO_2 incubator. Following incubation, 1 ml of growth medium containing twice the normal concentration of serum was added without removing the transfection mixture. The medium was replaced with fresh complete medium at 18 to 24 hours following start of transfection. Cells were active for gene activity 24 to 42 hours after the start of transfection.

Insertion of eGFP Gene into the Vector

The eGFP gene was cut out of the Clontech vector (GenBank Accession number U55761, Catalog number 6086-1) using Hind III and Not I at the multiple binding site region (Figure 15). The eGFP gene was then cloned into the Invitrogen vector (pc DNA1/Amp; 4.8kb) at the site just after the P cmv consitutative promoter using Hind III and Not I in accordance with the method as outlined in Figure 15, so that the final construct is as per represented in Figure 14.

Storage of Dermal Sheath Tissue

Cold temperature storage of dermal sheath tissue/cells; additionally their subjection to adverse conditions to highlight stem cell-type characteristics - including capacity for preferential survival.

Human skin samples (as detailed directly above) were cleaned and appropriately microdissected to provide: (a) $3mm^2$ portions of whole skin; (b) isolated hair follicles; (c) fragments of glassy membrane sandwiched between thin layers of sheath dermis and ORS epidermis, and (d) primary cultures of dermal sheath cells (prepared as above). Each of these four levels of tissue complexity were then subjected to six different forms of adverse conditions (each repeated with and without serum, and/or, glucose and glycerol): (i) prolonged cold temperature storage at 4^0C; (ii) repeated freeze/thaw cycles at -20^0C; (iii) repeated freeze/thaw storage at -80^0C in DMSO;

RESULTS

Sheath implants

All of the sites that had been implanted with dermal sheath tissue healed rapidly and in a manner that seemed typical of any superficial skin lesion. Fine narrow scabs formed as the site dried and then were lost over the next few days to leave a very faint wound, which was almost imperceivable by about the 10th day. There was no external sign of any inflammatory reaction in or around the wounds, nor any physical perception of the site. The tip of a fibre that was darker and disproportionately sturdier for its length than any of the arm skins local vellus hairs, was first noticed on the 24th day after the dermal sheath had been introduced. On the 33rd day post-implantation, a second much finer and unpigmented fibre was seen to have emerged just to the side of the first. A very light peppering of pigmented material was also visible below the surface of the skin, in the immediate vicinity of the healed sites. In addition, a dark line of material could be seen underneath the skin directly behind the base of the larger fibre (refer to Figures 4 and 5). This almost certainly represented a continuation of the exposed length of hair, and indicated that the follicle producing it was shallowly embedded and at an unusual angle and orientation relative to the local follicles. Both fibres increased in mass and length over the next few weeks, but this was more pronounced in the pigmented fibre which became more obviously stouter and thus morphologically distinct from the local hairs (refer to Figures 4 and 5). The finer white fibre was covered by a thin layer (or sac) of dried cells, but otherwise, was quite similar in stature and general appearance to the neighbouring non-induced hairs. Twenty one days after the second set of operations (initiated three months after the first) a fibre (again darker and sturdier than the local hairs) was seen at the sheath-implanted site. Over a further similar time span of three weeks, this solidly pigmented hair grew thicker and became more curved. The site was biopsied on day 42.

Histological examination of the sheath-implanted sites confirmed that the two larger follicles which had produced terminal-type fibres externally, had all of the characteristic components. For instance, large oval (Alcian blue-positive) dermal papillae (Fig. 6, legend P) were overlaid by a pigmented epidermal matrix, and follicle-typical concentric tissue layers could also be clearly seen in transverse sections. However, these follicles were quite different from the local vellus population in terms of their: larger size; shallow depth of growth within the skin, and unusual angle of orientation parallel to the skin surface. Such independent and contrasting features strongly suggest that the larger appendages were induced.

Notably, none of the transplanted material was transplanted into an immunoprotected site.

Further smaller follicles were also noted in random positions and arrangements in and around the post-experimental wound sites, and while they too may have been newly formed, their situation could not be interpreted on the basis of the morphological criteria alone.

Evidence in support of immunoprivilege as illustrated by in situ hybridisation

Both positive (refer to Figure 7 which represents pictorial evidence of a lower portion of an induced follicle which can be seen to stain positively following in-situ hybridisation with a Y-chromosome-specific DNA probe, realised via digoxygenin label) and negative (refer to Figure 8 which represents pictorial evidence of a tissue section acting as a negative control for Figure 7, and represents female skin that is not stained at all by the digoxygenin-linked Y-chromosome probe) controls stained appropriately to confirm the validity of the

protocols basic methodology.

In the first set of experimental tissue sections, both of the Y-chromosome-specific DNA probes recognised some of the smaller follicles in the wound sites, as well as the more predictably induced larger ones. Only the lowermost regions of the smaller follicles, in fact, little more than the end bulb regions, repeatedly stained positively with the probes (compare Figures 7 and 8), as visualised by either the digoxygenin or the Spectrum green fluorophore to indicate the cells of male origin. Unfortunately, the morphological resolution of the tissue was not adequate to interpret the probes distribution at the level of individual cells, or even tissue layers. Nevertheless, that both the fluorophore, (refer to Figure 9 compared to Figure 10) and digoxygenin - (Figures 7 compared to 8) labelled probe recognised almost identical regions of the follicles tissue as positive, was considered to reinforce the results.

Experimental Evidence in support of the ability of dermal sheath cells to provide long term replacement skin dermis

Dermal sheath cells were recombined with epidermal cells from hair follicles and grafted, inside a chamber that separated the graft from the surrounding skin cells, onto an animal.

The dermal sheath cells formed a very good dermis with uniform cell density and no sign of abnormal collagen formation. They also interacted with the epidermis to produce a thick epidermal covering. A complete and normal basement membrane was formed between dermal sheath and epidermis. Where the chamber surrounding the graft has been removed, the white block cell infiltrate that has built up outside the graft does not appear to enter the new skin site. Refer to Figure 11 which represents a high power magnification view of

the side of a long term [24 days] graft. The line of dark dense white blood cell infiltrate on the left, has not encroached into the graft site. In the dermis, collagen bundles are structured, dermal cells are regularly distributed and a complete and normal basement membrane is obvious.

Experimental Evidence in support of dermal sheath cell stem cell potential

Figure 12 (A-I) represent pictorial evidence of dermal sheath cells capability to differentiate into different mesenchymal cells and hence their stem cell potential. It can be seen that these cells can differentiate into myotubes, adipocytes, chondrocytes and mineral producing bone cells. Further surprising evidence includes hair follicle tissue, obtained from individuals in the 95-105 age range, was found to be viable and capable of acting as a productive source for cell culture initiation. This data supports the hypothesis of the capability of stem cells to differentiate and reproduce remains constant during lifetime (10). Additionally repeated freezing and thawing of primary dermal sheath cells and subsequent cloning did not alter their potential to exhibit at least four different phenotypes despite their prior exposure to adverse conditions.

Experimental Evidence in Support of Dermal Sheath Multipotentiality

Muscle myotubes

Subpopulations of small spindle-shaped cells were observed both singularly and in various states of fusion (as can also be commonly seen in routinely prepared cultures), some forming long branching, multinucleate myotube-like structures. A proportion of these cells strained positively with myosin, desmin and/or alpha-smooth muscle actin monoclonal antibodies. [There have even been an odd occasion in the past when we have observed spontaneous rhythmic beating,

i.e. contractions, of long aggregations of such muscle precursor-type cells in our petri dishes].

Adipocytes

These cells were identified by their distinctive multivesiculate appearance and the fact that the material contained within their vesicles was stained red by Sudan IV, and thus shown to be saturated neutral lipid.

Chondrocytes

Seen as accumulations of rounded cells with pericellular pH 1.0 Alcian Blue positive material which would be chondriotin and keratan sulphate proteoglycans, and lacunae between many of the cells - {interestingly similar cell behaviour is observed when rat dermal sheath cells are mixed with microdissected ear cartlidge in vitro}. This also seems likely to be related to our observations in vivo, when implanted dermal sheath cells appear to stimulate hyperplasia in the normally inactive ear cartilage.

Mineral producing Bone Cells

These cells were identified by their formation of aggregates in which the matrix appeared mineralised and stained positively for calcium phosphates, after being treated by the von Kossa method.

Further distinctive cell types have also been observed in our dermal sheath cell cultures (including interesting dendritic populations) but as yet these remain inaccurately defined.

Experimental Evidence in support of dermal sheath cells as substitutes for fibroblasts in skin wounding

Fluorescent dye (DiI) labelled dermal sheath cells and fibroblasts were implanted into skin wounds in a collagen gel, dermal sheath cells survived comparably to skin cells over 10 days and were observed to penetrate further into host skin. Dermal sheath cells were also shown to be capable of migration and incorporating themselves into normal skin away from the wound itself (refer to Fig. 13 which represents pictorial evidence of skin at the margin of a wound and in which dermal sheath cells have surrounded an isolated follicle remote in undamaged tissue).

Storage of Dermal Sheath Tissue

Our investigations have shown that dermal sheath tissue and/ or cells derived therefrom can be stored long term at low temperatures and yet still, when subjected to appropriate conditions, grow. This clearly has important implications in the storage of wound healing therapeutics, and specifically, the storage of grafts or "living skins" made therefrom.

Moreover, our investigations have also shown that the dermal sheath cells can persist for a long time in culture under conditions of extreme stress. This has important implications for wound healing therapeutics derived from this tissue, since it highlights that it is favourably robust and also that it displays stem cell characteristic durability and viability.

Evidence in Support of Transfected Dermal Sheath Cells

Using the construct containing enhanced green fluorescent protein e-GFP

depicted in Figure 14 obtained by the method outlined in Figure 15. Two sets of dermal sheath cells were transfected on two separate occasions and were shown to visibly express the GFP by 36 hours (refer to Figure 16). Any cells that contained the construct were identified by their fluorescence. Transfection rates were reasonably high, approaching 20% of the cells. Furthermore, the cells remained green for more than 2 weeks. It is our assumption that the cells would survive in vivo if they were put back into humans/other species either at the same or different sites.

In short, not only does dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles have all the advantageous properties that one might hope to find in a gene therapy system but they also have properties that facilitate the use of the tissue and/or cells derived therefrom in terms of manufacturing and long term storage.

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CLAIMS

1. Dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles for use in gene therapy.

2. A gene therapy vehicle for delivering at least one selected gene, or functional fragment thereof, to a target site comprising dermal sheath tissues and/or cells derived therefrom and/or cells typically closely associated with hair follicles.

3. Dermal sheath tissue or a gene therapy vehicle according to Claims 1 or 2 wherein said tissue or cells is/are derived from the lower portion of a hair follicle.

4. Dermal sheath tissue or a gene therapy unit according to Claim 3 wherein said tissue or cells are derived from a lower third of said hair follicle.

5. Dermal sheath tissue or a gene therapy unit according to Claim 3 or 4 where said tissue or cells are derived from a segment or ring of a combination of follicle/tissue cells.

6. A gene therapy vehicle according to Claims 2-5 which is suitably engineered by recombinant techniques so as to include at least one insertion site into which at least one selected gene can be placed.

7. A gene therapy vehicle according to Claims 2-6 wherein said selected gene is functionally inserted into said gene therapy vehicle so that the expression of said selected gene results in the provision of the corresponding protein product.

8. A gene therapy vehicle according to Claims 2-7 wherein said vehicle is provided with multiple insertion sites to carry multiple genes and so provide for the delivery of multiple proteins.

9. A gene therapy vehicle according to Claim 8 wherein said multiple proteins are of a similar nature.

10. A gene therapy vehicle according to Claim 8 wherein said multiple proteins are of a different nature.

11. A gene therapy vehicle according to Claims 2-10 wherein said selected gene for insertion is arranged so as to be inserted in frame with the genome of the gene therapy vehicle so as to provide for correct expression of said selected gene.

12. A gene therapy vehicle according to Claims 2-11 wherein said selected gene is operationally linked to a regulatable promoter.

13. A gene therapy vehicle according to Claims 2-11 wherein said selected gene is operationally linked to an inducible promoter.

14. A gene therapy vehicle according to Claims 2-11 comprising wherein said selected gene, is operationally linked to a constitutive promoter.

15. A vector for transforming or transfecting the gene therapy vehicle of Claims 2-14 wherein said vector is provided with at least one insertion site into which at least one selected gene, or functional fragment thereof, can be placed and also other expression control elements for ensuring that once the vector infects or penetrates said tissue and/or cells of said gene therapy vehicle, expression of said selected gene can take place.

16. A therapeutic composition comprising a suitable carrier and the gene therapy vehicle according to Claims 2-14.

17. A therapeutic composition according to Claim 16 wherein said composition is formulated to have anti-bacterial properties.

18. A therapeutic composition according to Claim 16 or 17 wherein said composition is formulated to have anti-septic properties.

19. A therapeutic composition according to Claims 16 - 18 wherein said composition is formulated to include growth promoting additives.

20. A therapeutic composition according to Claims 16-19 wherein said composition includes at least one anaesthetic.

21. A therapeutic composition according to Claims 16-20 wherein said composition is adapted to be applied topically in the form of dermal sheath cells provided in a suitable carrier solution, gel, cream or emollient.

22. A therapeutic composition according to Claims 16-20 wherein said

composition is adapted to be administered by injection and so comprises a carrier solution.

23. A therapeutic appliance comprises a therapeutic composition according to Claims 16-22 wherein said carrier is incorporated and/or embedded therein, and/or associated therewith, and/or attached thereto, a plaster or bandage.

24. A gene therapy vehicle for use in delivering a selected gene, or functional fragment thereof, to a given site wherein said gene therapy vehicle comprises dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles, which tissue and/or cell have been suitably adapted to accommodate heterologous genetic material and which, in vivo, have the capacity to selectively differentiate to provide at least one differentiated tissue type.

25. A gene therapy vehicle according to Claims 2-14 and 24 which is adapted to be provided as a wound healing system.

26. A wound healing system comprising a suitable matrix material having incorporated and/or embedded therein, and/or associated therewith, and/or attached thereto, a gene therapy vehicle according to Claims 2-14 and 24.

27 A wound healing system according to Claims 26 wherein said matrix material comprises native collagen.

28. A wound healing system according to Claims 26 or 27 wherein said matrix material comprises collagenous gels or lattices constructed from reconstituted collagen or highly complex mixtures of reconstructed collagen.

29. A wound healing system according to Claims 26-28 wherein said matrix material comprises extra cellular matrix products.

30. A wound healing system according to Claims 26-29 comprising a surgical dressing.

31. A wound healing system according to Claims 26-30 adapted for use in the treatment of acute, and/or chronic, and/or minor, and/or severe, wound healing.

32 A wound healing system according to Claims 26-31 for use in the treatment of cartilage repair, and/or bone repair, and/or muscle repair, and/or connective tissue repair, and/or blood vessel repair.

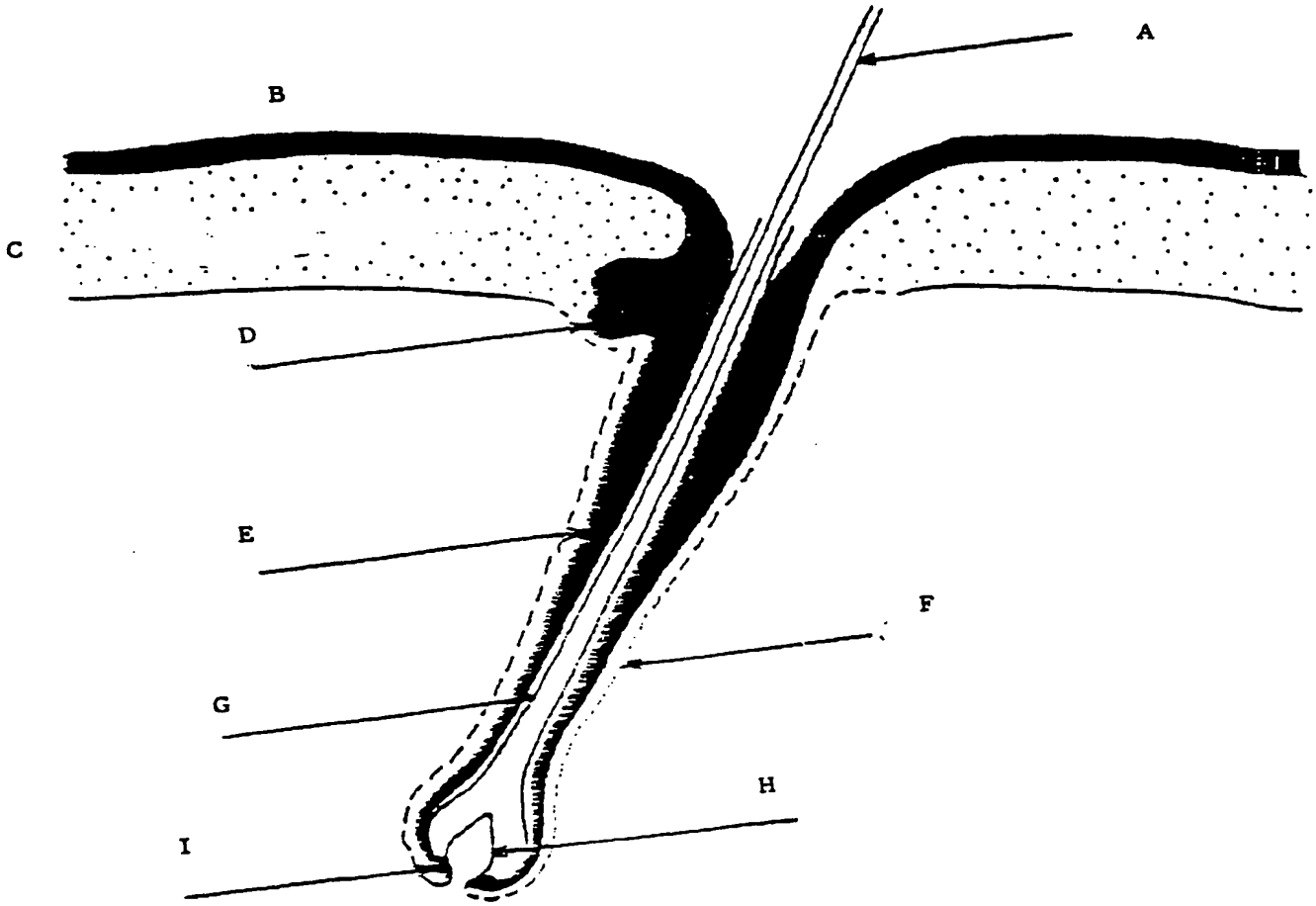
33. A wound healing system according to Claims 26-32 wherein said system comprises a plurality of cell types from a hair follicle.

34 A wound healing system according to Claim 33 wherein one of said cell types, in addition to said dermal sheath tissue, and/or cells derived therefrom, and/or cells typically closely associated with hair follicles, comprises dermal papilla tissue.

35. A therapeutic composition according to Claims 16-23 wherein said composition comprises a plurality of cell types from a hair follicle.

36. A therapeutic composition according to Claim 34 wherein one of said cell types, in addition to said dermal sheath tissue, and/or cells derived therefrom, and/or cells typically closely associated with hair follicles, comprises dermal papilla tissue.

Figure 1



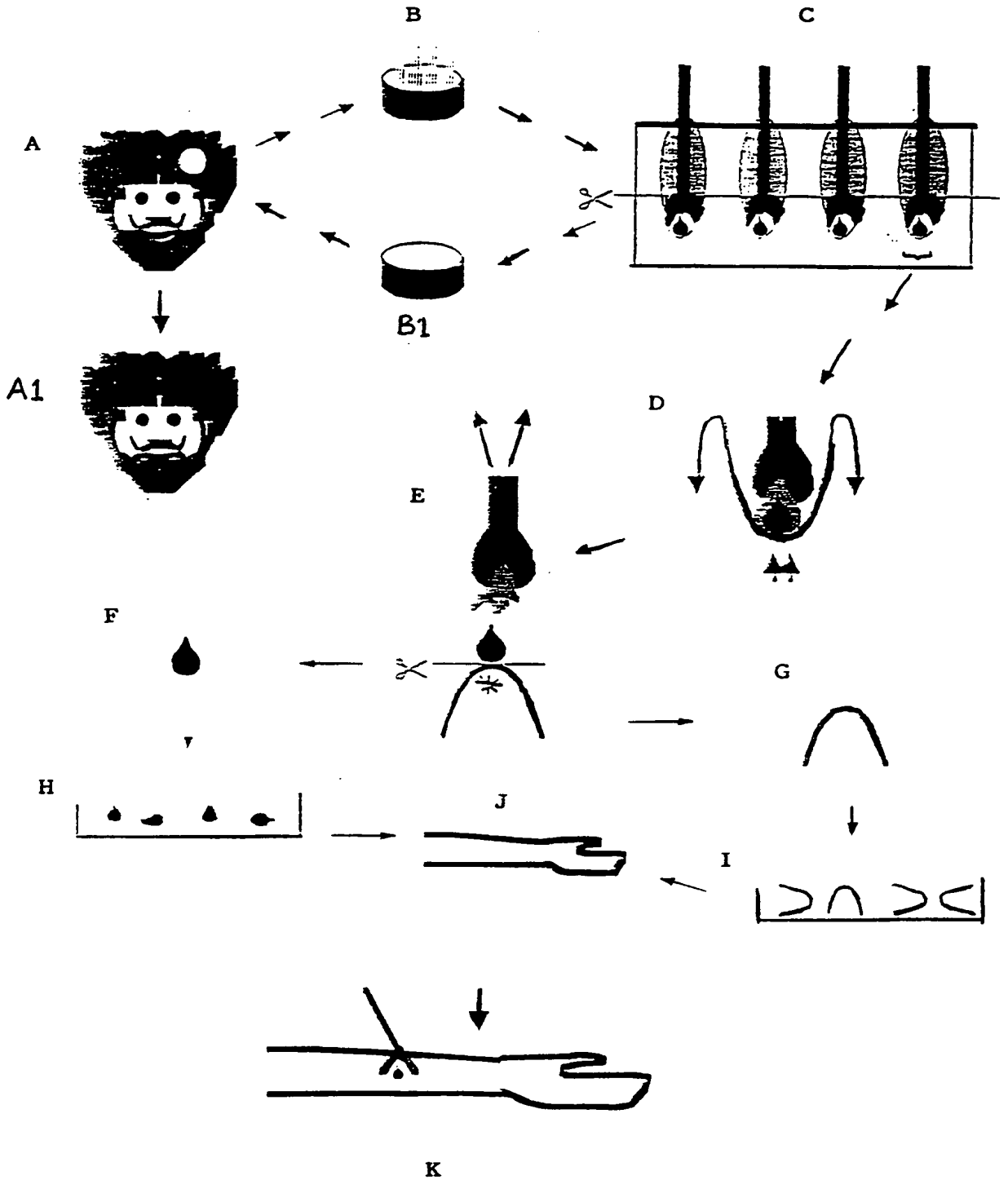

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FIG. 3

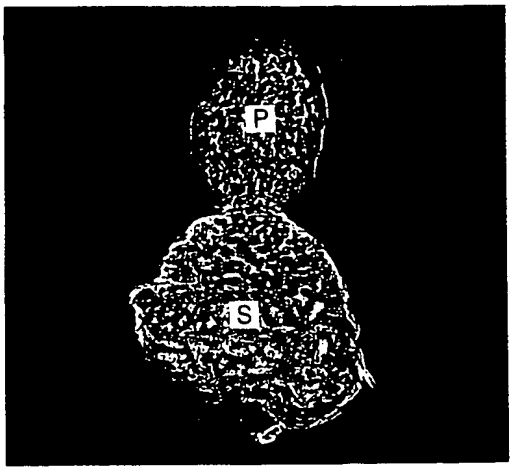


Fig. 4

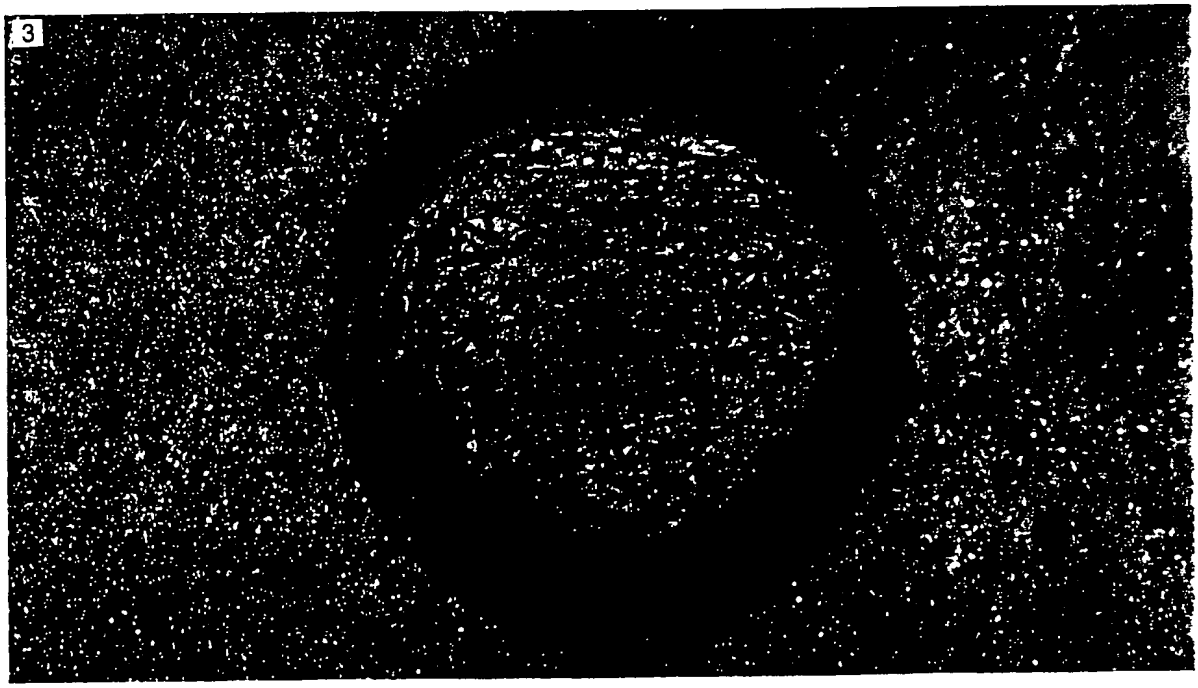


Fig 5

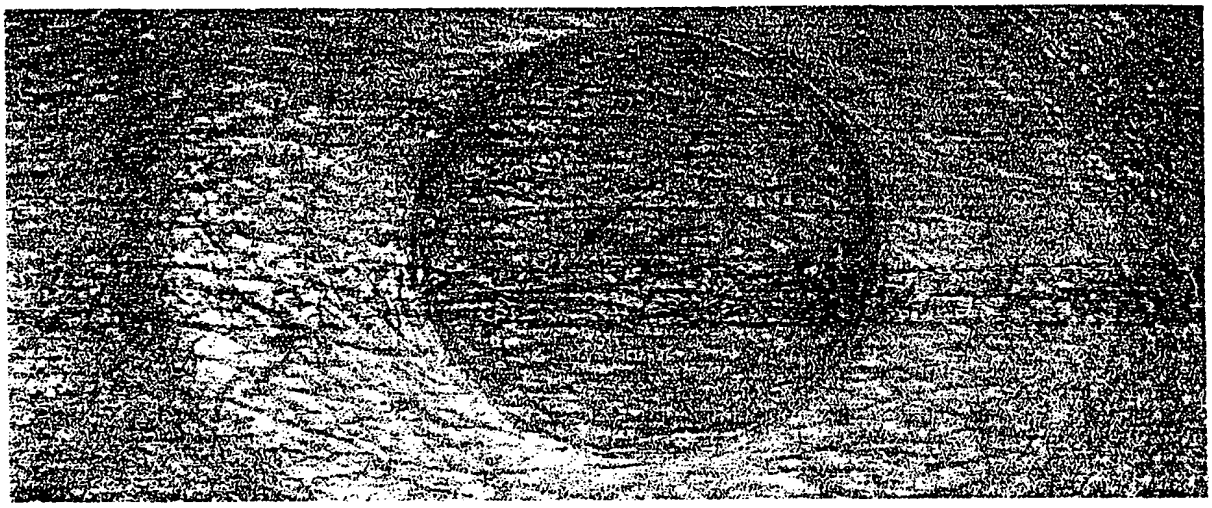


Fig 6

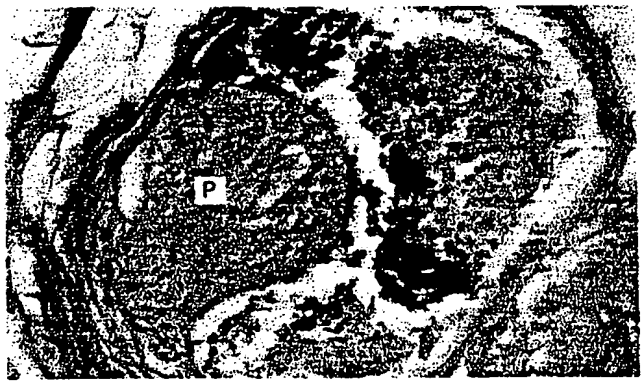


Fig 7

Fig 8

Fig 9

Fig 10

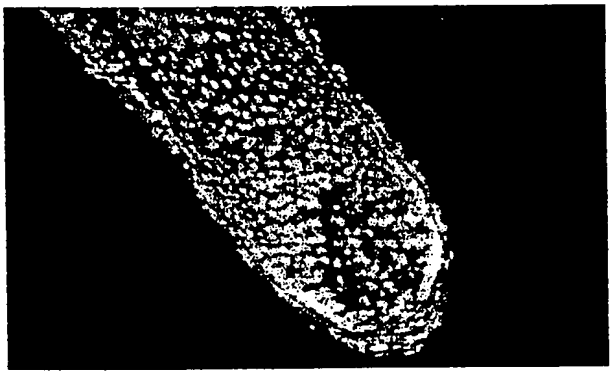


Fig 11

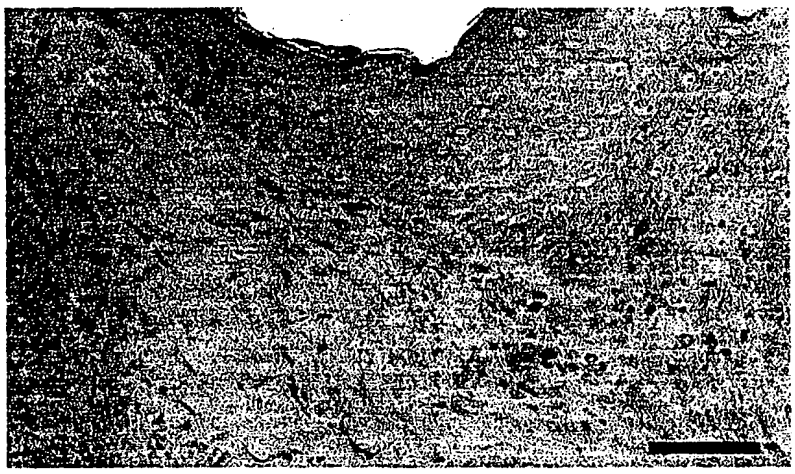


Fig 12 A

Fig 12 B

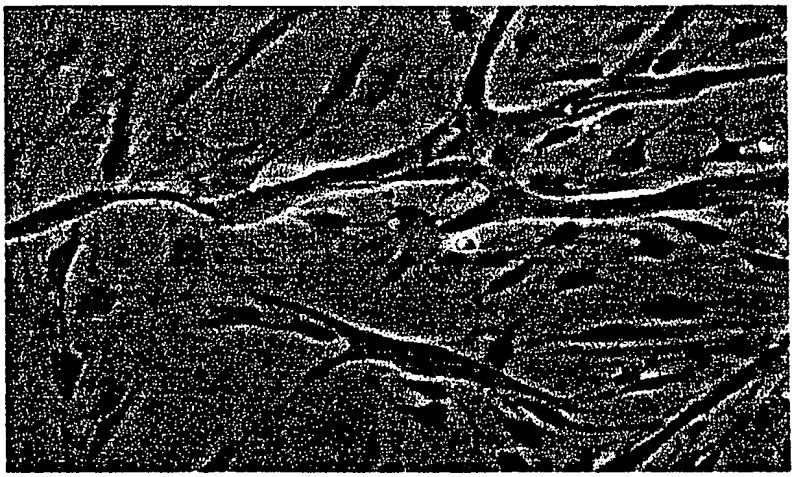


Fig 12C

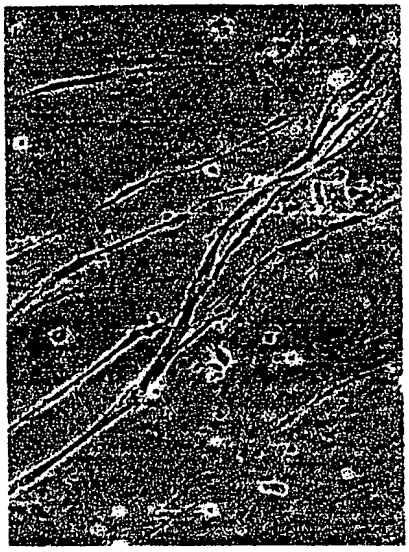


Fig 12D

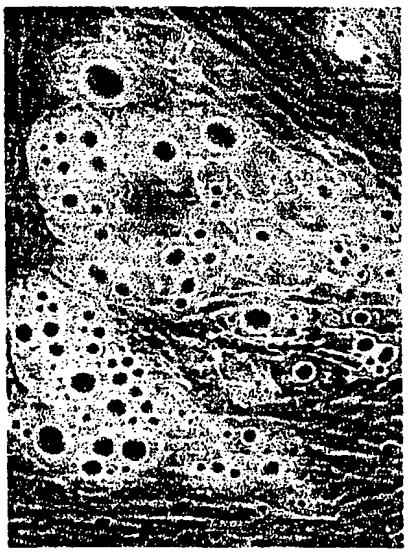


Fig 12E

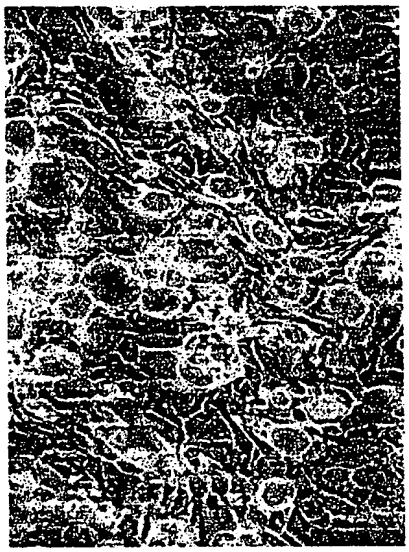


Fig 12F

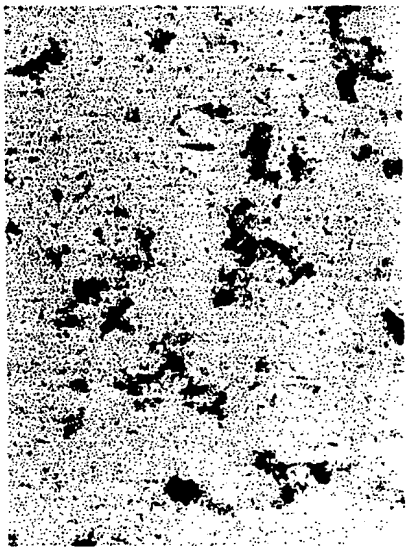


Fig 12G

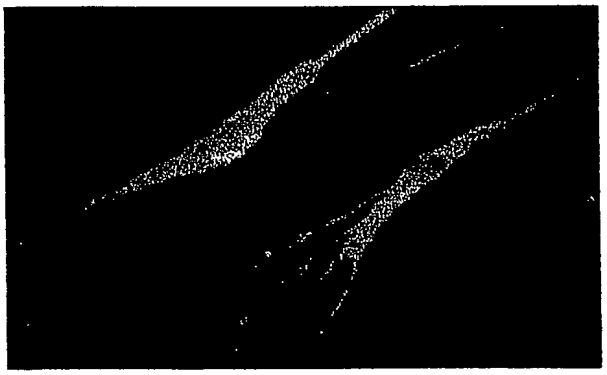


Fig 12H

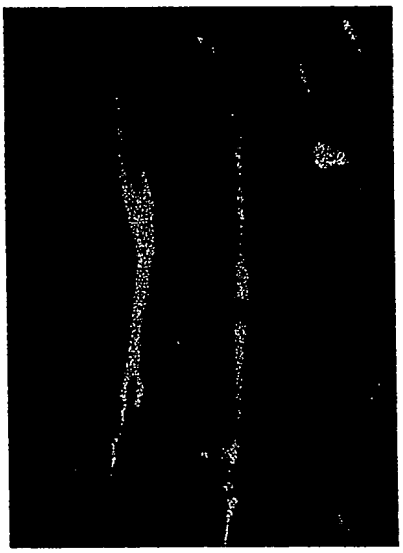


Fig 12 I

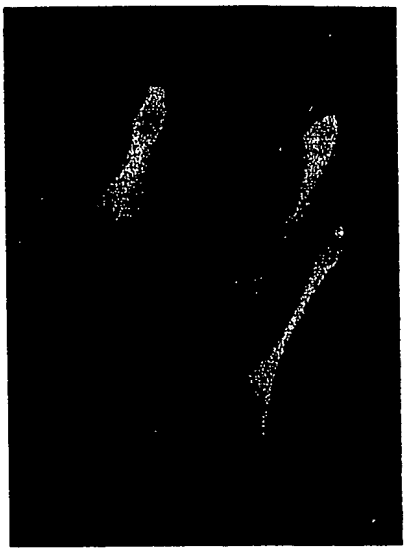


Fig 13

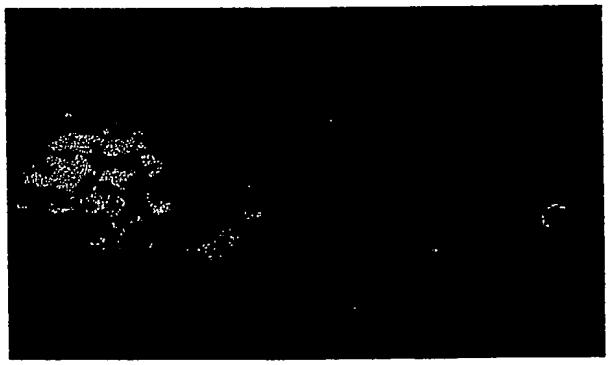
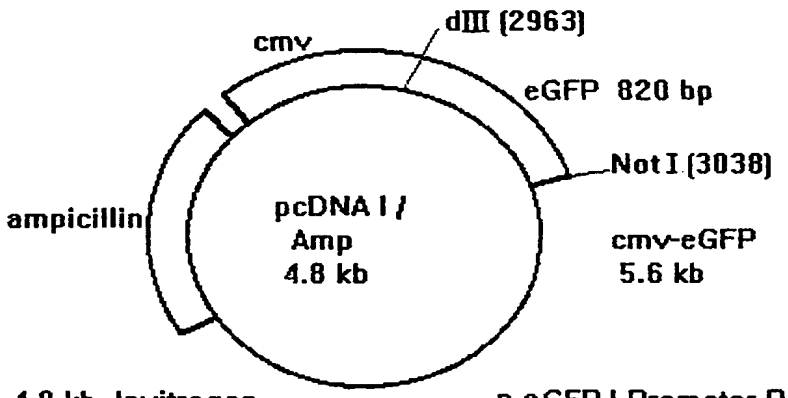


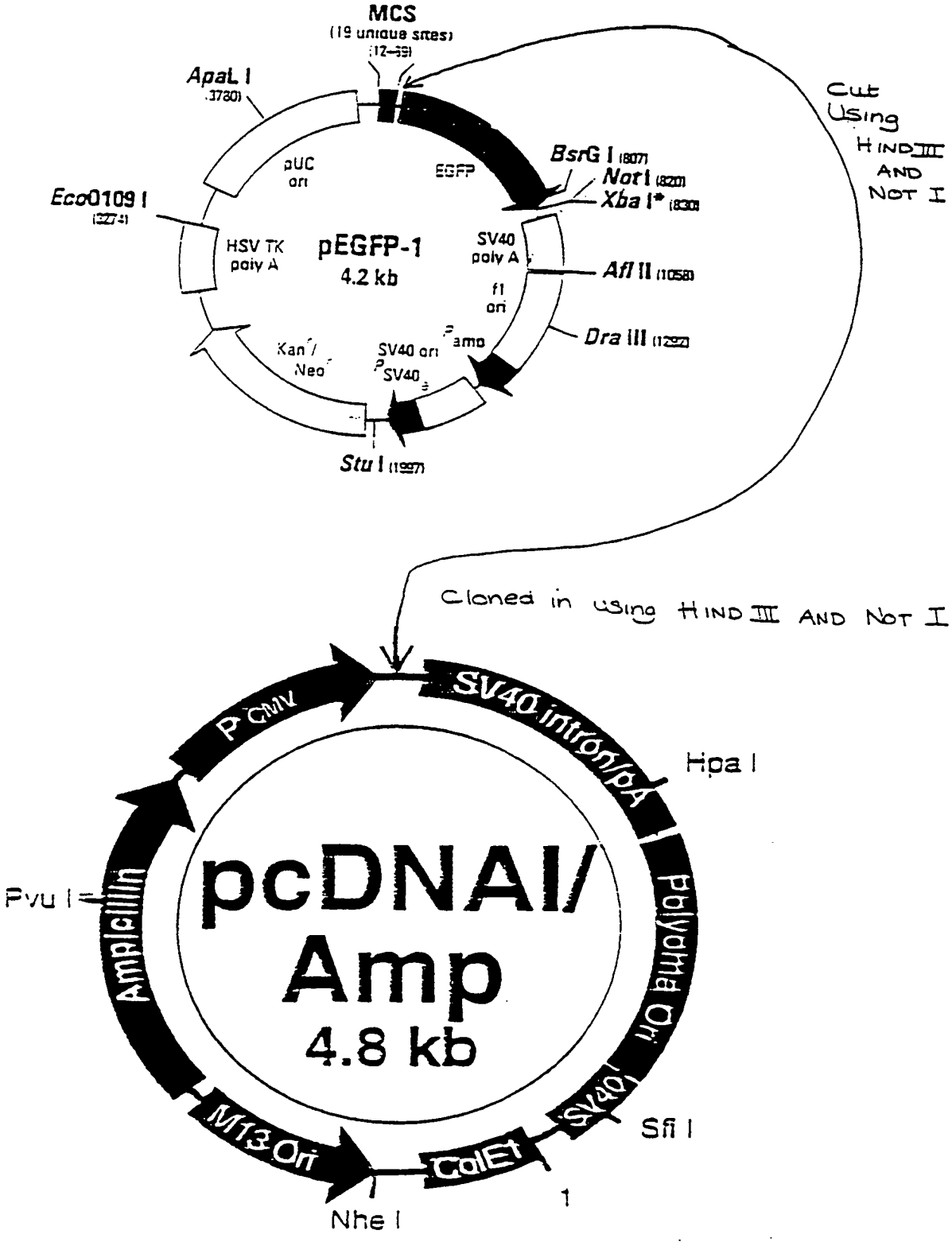
Figure 14.



pcDNA I / Amp 4.8 kb Invitrogen supercoiled plasmid

p eGFP-I Promoter Report r Vector 4.2 kb Clontech

eGFP gene removed via cutting with Hind III (41) and Not I (820)


MCS
(19 unique sites)
(12–33)
ApaL I
(3780)
pUC
ori
EGFP
BsrG I (807)
Not I (820)
Xba I* (830)
Eco0109 I
(3274)
HSV TK
poly A
pEGFP-1
4.2 kb
SV40
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Afl II (1058)
f1
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Dra III (1292)
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Polyoma Ori
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Nhe I
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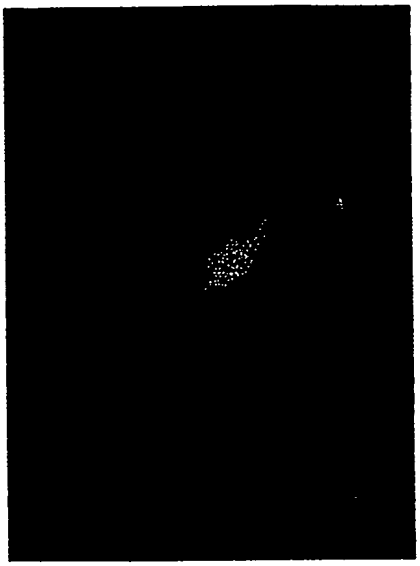


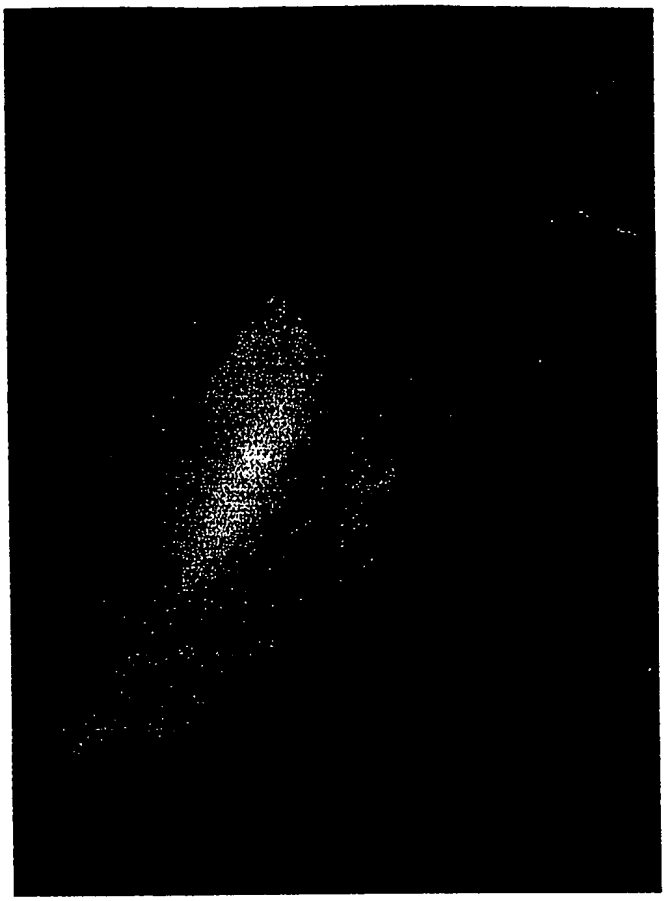
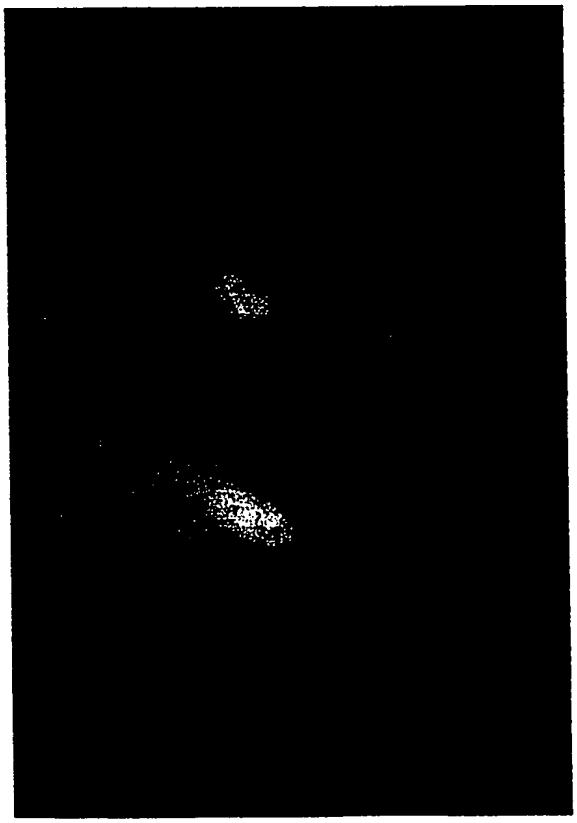
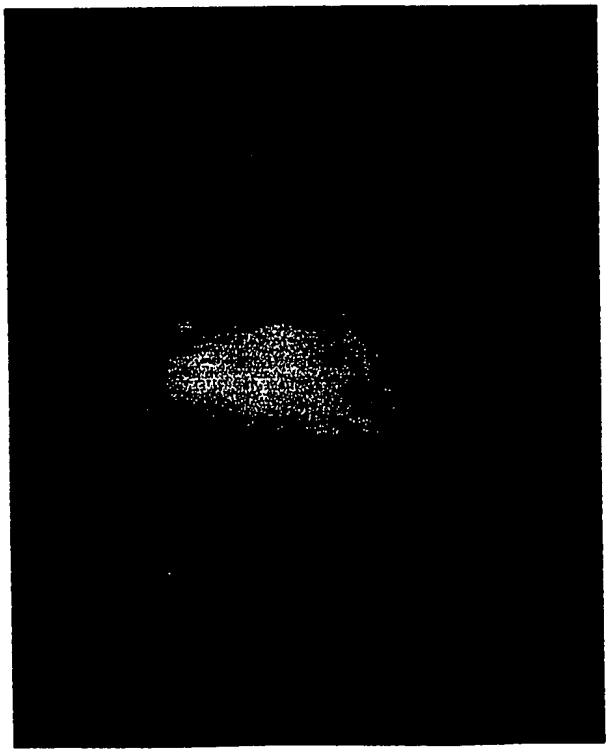


Fig.16





INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02150

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K48/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 25660 A (BRIGHAM & WOMENS HOSPITAL) 23 December 1993 see claims	1-36
X	EP 0 679 402 A (JAPAN RES DEV CORP ;YOSHIDA SUSUMU (JP); CUTHBERTSON R ANDREW (US)) 2 November 1995 see page 3, line 9 - line 20	1-36
X	WO 89 02468 A (WHITEHEAD BIOMEDICAL INST ;HOWARD HUGUES MEDICAL INST (US)) 23 March 1989 see claims	1-36
X	GB 2 293 604 A (BRITISH TECH GROUP) 3 April 1996 see example 6	1-36

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 1998	01/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02150

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 22430 A (BAYLOR COLLEGE MEDICINE ;UNITED SATES OF AMERICA (US)) 11 November 1993 see claims	1-36
X	EP 0 236 014 A (UNIV DUNDEE) 9 September 1987 see claims	1-5, 11-14, 16,19, 21,22, 25,26, 31-36
X	WO 95 01423 A (UNIV PENNSYLVANIA ;UNIV NEW YORK (US)) 12 January 1995 see claims	1,2,5,8, 11-14, 16,21, 22,25, 26,31,32
A	MOLL I.: "Proliferative Potential of Different Keratinocytes of Plucked Human Hair Follicles" JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 105, no. 1, 1995, pages 14-21, XP002084482	1-36

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/02150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9325660 A	23-12-1993	US 5423778 A	13-06-1995
		AU 4535693 A	04-01-1994
		EP 0644929 A	29-03-1995
		JP 8509356 T	08-10-1996
		US 5661132 A	26-08-1997
		US 5697901 A	16-12-1997
EP 0679402 A	02-11-1995	AU 1766595 A	02-11-1995
		CA 2147810 A	26-10-1995
		JP 8196271 A	06-08-1996
WO 8902468 A	23-03-1989	AT 117375 T	15-02-1995
		DE 3852823 D	02-03-1995
		DE 3852823 T	24-05-1995
		EP 0378576 A	25-07-1990
		EP 0633318 A	11-01-1995
		JP 3500124 T	17-01-1991
		US 5460959 A	24-10-1995
GB 2293604 A	03-04-1996	AU 694957 B	06-08-1998
		AU 3481695 A	09-04-1996
		CA 2198379 A	28-03-1996
		EP 0783568 A	16-07-1997
		WO 9609373 A	28-03-1996
		JP 10505756 T	09-06-1998
WO 9322430 A	11-11-1993	AU 4220793 A	29-11-1993
		CA 2134675 A	11-11-1993
		EP 0652948 A	17-05-1995
		JP 8503844 T	30-04-1996
EP 0236014 A	09-09-1987	AU 598235 B	21-06-1990
		AU 6915187 A	27-08-1987
		CA 1306416 A	18-08-1992
		DE 3771747 A	05-09-1991
		GR 3002975 T	25-01-1993
		JP 62246508 A	27-10-1987
		US 4919664 A	24-04-1990
WO 9501423 A	12-01-1995	US 5556783 A	17-09-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter: nal Application No
PCT/GB 98/02150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9501423 A		AU 7213294 A	24-01-1995
		US 5756094 A	26-05-1998

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Our ref: **FP2400**

BY FAX AND POST TO: 00 49 89 2399 4465

European Patent Office
D-80298
MUNICH
Germany

For the attention of: **The International Preliminary Examining Authority**

17 June 1999

Dear Sirs

International Patent Application No. PCT/GB 98/02150
In the name of: REYNOLDS-JAHODA, Amanda et al
Gene Therapy Vehicle

In response to the Written Opinion dated **21 April 1999** we file herewith to overcome the objections of the Examiner comments in response.

Amendments and Basis for Amendments

Claims 1, 2 and 24 have been amended to more clearly define the subject matter to which the present application relates. The invention relates to genetically engineered dermal sheath cells/tissue. Basis for this amendment can be found on page 6, line 16- page 7, line 21; Figure 2 and "Tissue Isolation" on page 18 of materials and methods.

Novelty

We respectfully submit new Figure 1, which has been coloured to demarcate the origins of dermal sheath cells/tissue, which is the subject of the present application. It is evident from new Figure 1 and the disclosure in the description and drawings, that the dermal sheath cells/tissue of the present application are distinct from the cells/tissues described in citations D1-D8 (which have also been demarcated on new colour figure 1).

With respect to the cited prior art we have the following comments.

17 June 1999

D1: WA 93/25660

This document describes, amongst other things, transfection of **keratinocytes** and/or **epidermal stem cells** (see new figure 1) with viral vectors or plasmids and their use in wound healing.

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and therefore cannot be considered detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D2: XP002084482 Journal of Investigative Dermatology

This document describes **outer root sheath keratinocytes** isolated from human, plucked, hair follicles. Referring to new Figure 1, this does not relate to the dermal sheath cells/tissue of the present application. The cells of the present application require microdissection (please see new Figure 1, Figure 2 and page 17, " Tissue Isolation") and are a select population of cells.

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and therefore cannot be considered detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D3: A O 679 402

This document describes the use of genetically engineered **epidermal or fibroblast cells fr m interfollicular skin** (please see new colour Figure 1) containing an expression vector encoding a therapeutic gene (e.g. insulin) and their incorporation into a biopolymeric gel and in their use in gene therapy by skin transplantation.

This therefore relates to a quite distinct group of cells and does not disclose the use of genetically engineered dermal sheath cells/tissues for use in wound healing and cannot be considered detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D4: WO89/02468 A

This document describes genetically engineered **fibroblasts, interfollicular mesenchymal or connective tissue cells** (please see new colour Figure 1). The genetically engineered cells contain vectors expressing therapeutic genes for use in transplantation in skin grafts to provide a continues supply of said therapeutic gene product to an individual into which the genetically engineered cells have been introduced.

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and therefore cannot be considered detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D5: GB A 2293 604

This document discloses the use of genetically engineered **dermal fibroblasts** (please see new colour Figure 1) **derived** from neonatal mice in gene therapy, specifically the provision of dermal fibroblasts expressing the muscle specific gene, dystrophin, as a means to treat genetic disorders such as muscular dystrophy.

17 June 1999

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and there e cannot be considered a detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D6:WO A 93 22430

This document discloses the use of genetically engineered **epidermal cells** (please see new colour Figure 1) and their use in gene therapy, specifically the provision of epidermal cells expressing a vector engineered to contain control sequences of the keratin K1 gene to confer epidermal specific expression on genes subcloned into said vector for use in the treatment of various disorders (e.g. skin ulcers, wound healing, surgical incisions, psoriasis and cancer)

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and therefore cannot be considered a detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

D7: EP O 236 014

This document relates to **follicle dermal papilla cells** (shown in green in new colour Figure 1) and their use in the stimulation of hair growth. There is no disclosure of the use of such cells in wound healing nor are these cells genetically engineered.

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and there for cannot be considered a detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

Furthermore, the Examiner's comment that Claims 1, 2, 16 and 26 are not restricted to genetically modified material is incorrect. Gene therapy has a generic meaning in the art and refers to the genetic modification of a cell/tissue/organism by transfer of genetic material into same.

D8: WO 95/ 01423

This document discloses a method for growing **hair follicle epidermal stem cells** (please see new colour Figure 1) from the upper portion of a hair follicle. This therefore relates to a quite distinct population of cells, which in addition are not genetically engineered.

This does not relate to the use of genetically engineered dermal sheath cells/tissue in wound healing and there for cannot be considered a detrimental to novelty of Claims 1 – 26, 30 – 33 and 35.

Furthermore, the Examiner's comment that Claims 1, 2, 16 and 26 are not restricted to genetically modified material is incorrect. Gene therapy has a generic meaning in the art and refers to the genetic modification of a cell/tissue/organism by transfer of genetic material into same.

Inventive Step

The Examiner considers Claims 1 – 36 lack inventive step.

17 June 1999

We respectfully submit that since the present application relates to a distinct population of cells which have immune privilege and the potential to differentiate into a number of different skin cell types and moreover this population of cells is not described in any of the cited documents D1 – D8 we consider that the use of genetically engineered dermal sheath tissue/cells would not be obvious to the man skilled in the art using the information disclosed in documents D1-D8.

We submit that Claims 1 –36 therefore are inventive over citations D1- D8.

Telephone Interview

In view of the above argumentation we now expect to receive a favourable International Preliminary Examination Report with respect to novelty and inventive step of Claims 1 – 36. If the Examiner has any remaining doubt as to novelty and inventive step they are requested to telephone Dr Rob Docherty for a telephone interview (Rule 66.6 PCT).

Please return the enclosed EPO Form 1037 as acknowledgement of receipt.

Yours faithfully

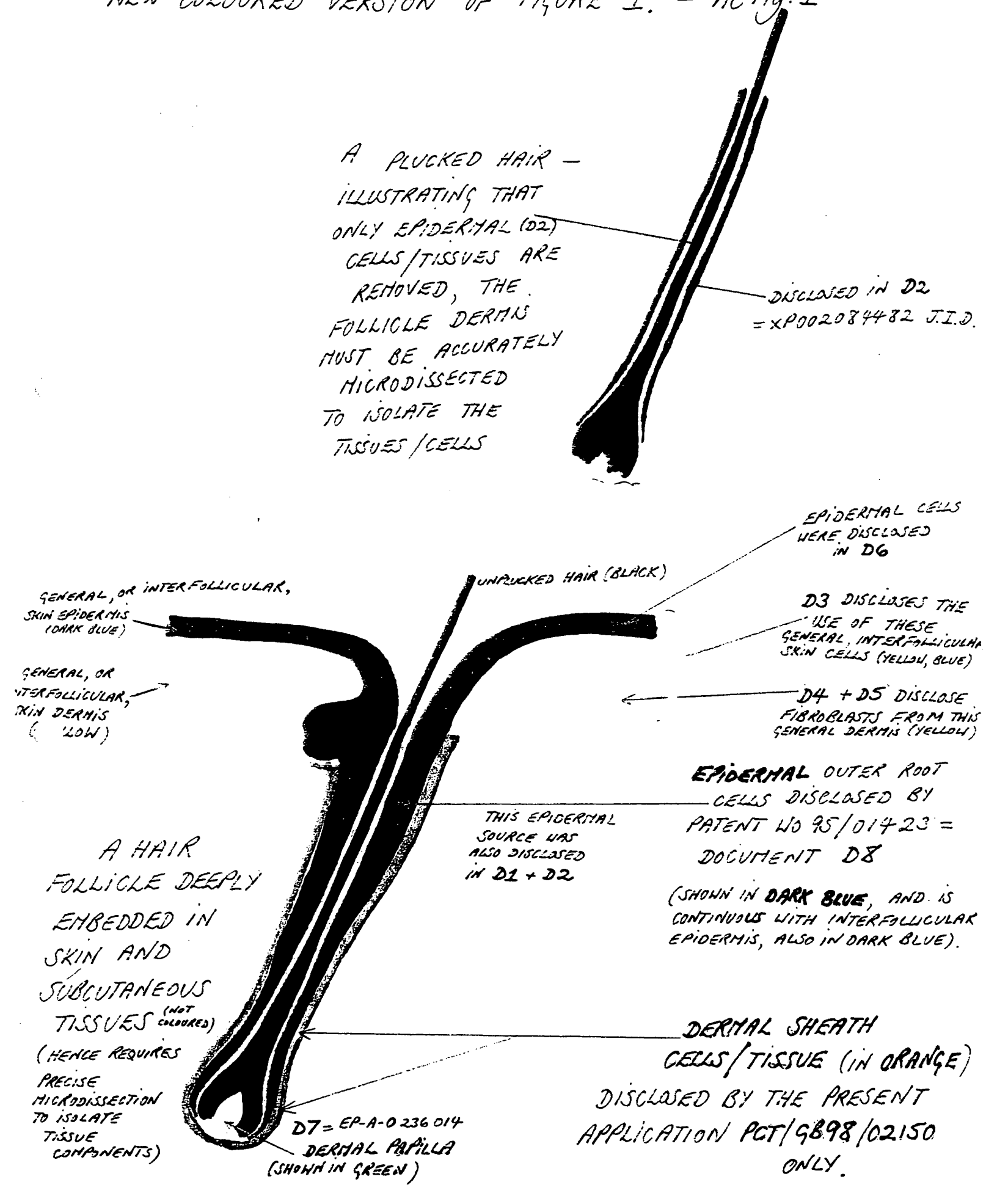
MARKGRAAF PATENTS LIMITED

Encs: Amended Claims 1-36 [x3]
New Figure 1
Form 1037.1

RCD/ne/ab

Sent with letter to EPO 17/6/99 (42)

NEW COLOURED VERSION OF FIGURE 1. = nc Fig. 1



PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

03 MAR 99

To:
MARKGRAAF PATENTS LIMITED
Crescent
54 Blossom Street
York Y024 1AP
GRANDE BRETAGNE

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	01.03.99
Applicant's or agent's file reference: FP2400	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 98/02150	17/07/1998	18/07/1997

Applicant: REYNOLDS-JAHODA, Amanda et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   09/02/1999

2. This date of receipt is:

   - [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
   - [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
   - [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

   [ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	P. Donnelly Pamela Donnelly Telephone No.

PATENT COOPERATION TREATY

17. NOV 99

18.NOV.1999*020878 YORK

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MARKGRAAF PATENTS LIMITED
The Crescent
54 Blossom Street
York Y024 1AP
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	15.11.99
Applicant's or agent's file reference FP2400 P38038WO / RCD	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02150	17/07/1998	18/07/1997

Applicant
REYNOLDS-JAHODA, Amanda et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Senkel, H Tel.+49 89 2399-8071

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREA. .

PCT

18

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
FP2400		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02150	17/07/1998	18/07/1997
International Patent Classification (IPC) or national classification and IPC A61K48/00		
Applicant REYNOLDS-JAHODA, Amanda et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/02/1999	15.11.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Seegert, K Telephone No. +49 89 2399 8409

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02150

I. Basis of th r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-30	as originally filed				

Claims, No.:

1-36	as received on	21/06/1999	with letter of	17/06/1999	

Drawings, sheets:

1/11-11/11	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14,16-36
	No:	Claims	15
Inventive step (IS)	Yes:	Claims	1-14,16-36
	No:	Claims	15
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Section V

1. Reference is made to the following documents:

   D1: WO-A-9 325 660
   D2: Journal of Investigative Dermatology, vol 105 (1), 1995, pages 14 - 21
   D3: EP-A-0 679 402
   D4: WO-A-8 902 468
   D5: GB-A-2 293 604
   D6: WO-A-9 322 430

2. The subject-matter of the present application basically relates to dermal sheath tissue and/or cells derived therefrom for use in gene therapy.

3. Document D1 (e.g. claims) discloses a method for in vivo gene transfer into keratinocytes or follicular stem cells by using viral vectors or plasmids. The genetically modified cells are used e.g. for the healing of wounds.

   Similarly, document D2 (page 20, left-hand column, last sentence) suggests that keratinocytes derived from hair follicles can be successfully used in gene therapy.

   Document D3 (page 3, lines 9 - 20) discloses a hybrid gel for external use comprising genetically modified skin cells (epidermal or fibroblast cells) containing an expression vector encoding a biologically active substance optionally in conjunction with an antibiotic resistance gene.

   Similarly, documents D4 (claims) and D5 (e.g. example 6) disclose transduced fibroblasts for use in gene therapy, e.g. as a skin graft.

   Document D6 (claims) discloses the use of genetically transformed epidermal cells for use in e.g. wound treatment.

4. The subject-matter of claims 1 - 14 and 16 - 36 meets the requirements of the PCT with respect to novelty and inventive step (Article 33 (2), (3) PCT). The use

of dermal sheath tissue in gene therapy or in any other therapy had not been disclosed in the prior art (see analysis of D1 - D6 above). Although the prior art discloses the use of different parts of the hair follicle for the use in gene therapy, it would not have been obvious to the skilled person to use the specific part as disclosed in the present application.

5. As for claim 15 it is noted that the vector according to the present application seems to be commercially available (see page 22 of the description). Consequently, claim 15 is deprived of novelty. Vectors covered by the scope of claim 15 seem further to be disclosed in e.g. document D3 - D6.

Section VIII

1. The subject-matter of claims 1 and 2 lacks clarity within the meaning of Article 6 PCT since the claims do not contain every important feature in order to carry out the invention. In particular, the claims do not specify that the dermal sheath tissue/cell has to be genetically modified (see e.g. claim 6).

CLAIMS

1. Dermal sheath tissue and/or cells derived therefrom for use in gene therapy.

2. A gene therapy vehicle for delivering at least one selected gene, or functional fragment thereof, to a target site comprising dermal sheath tissues and/or cells derived therefrom.

3. Dermal sheath tissue or a gene therapy vehicle according to Claims 1 or 2 wherein said tissue or cells is/are derived from the lower portion of a hair follicle.

4. Dermal sheath tissue or a gene therapy unit according to Claim 3 wherein said tissue or cells are derived from a lower third of said hair follicle.

5. Dermal sheath tissue or a gene therapy unit according to Claim 3 or 4 where said tissue or cells are derived from a segment or ring of a combination of follicle/tissue cells.

6. A gene therapy vehicle according to Claims 2-5 which is suitably engineered by recombinant techniques so as to include at least one insertion site into which at least one selected gene can be placed.

7. A gene therapy vehicle according to Claims 2-6 wherein said

AMENDED SHEET

selected gene is functionally inserted into said gene therapy vehicle so that the expression of said selected gene results in the provision of the corresponding protein product.

8. A gene therapy vehicle according to Claims 2-7 wherein said vehicle is provided with multiple insertion sites to carry multiple genes and so provide for the delivery of multiple proteins.

9. A gene therapy vehicle according to Claim 8 wherein said multiple proteins are of a similar nature.

10. A gene therapy vehicle according to Claim 8 wherein said multiple proteins are of a different nature.

11. A gene therapy vehicle according to Claims 2-10 wherein said selected gene for insertion is arranged so as to be inserted in frame with the genome of the gene therapy vehicle so as to provide for correct expression of said selected gene.

12. A gene therapy vehicle according to Claims 2-11 wherein said selected gene is operationally linked to a regulatable promoter.

13. A gene therapy vehicle according to Claims 2-11 wherein said selected gene is operationally linked to an inducible promoter.

14. A gene therapy vehicle according to Claims 2-11 comprising wherein said selected gene, is operationally linked to a constitutive promoter.

15. A vector for transforming or transfecting the gene therapy vehicle of Claims 2-14 wherein said vector is provided with at least one insertion site into which at least one selected gene, or functional fragment thereof, can be placed and also other expression control elements for ensuring that once the vector infects or penetrates said tissue and/or cells of said gene therapy vehicle, expression of said selected gene can take place.

16. A therapeutic composition comprising a suitable carrier and the gene therapy vehicle according to Claims 2-14.

17. A therapeutic composition according to Claim 16 wherein said composition is formulated to have anti-bacterial properties.

18. A therapeutic composition according to Claim 16 or 17 wherein said composition is formulated to have anti-septic properties.

19. A therapeutic composition according to Claims 16 - 18 wherein said composition is formulated to include growth promoting additives.

20. A therapeutic composition according to Claims 16-19 wherein said composition includes at least one anaesthetic.

21. A therapeutic composition according to Claims 16-20 wherein said composition is adapted to be applied topically in the form of dermal sheath cells provided in a suitable carrier solution, gel, cream or emollient.

22. A therapeutic composition according to Claims 16-20 wherein said

composition is adapted to be administered by injection and so comprises a carrier solution.

23. A therapeutic appliance comprises a therapeutic composition according to Claims 16-22 wherein said carrier is incorporated and/or embedded therein, and/or associated therewith, and/or attached thereto, a plaster or bandage.

24. A gene therapy vehicle for use in delivering a selected gene, or functional fragment thereof, to a given site wherein said gene therapy vehicle comprises dermal sheath tissue and/or cells derived therefrom, which tissue and/or cells have been suitably adapted to accommodate heterologous genetic material and which, in vivo, have the capacity to selectively differentiate to provide at least one differentiated tissue type.

25. A gene therapy vehicle according to Claims 2-14 and 24 which is adapted to be provided as a wound healing system.

26. A wound healing system comprising a suitable matrix material having incorporated and/or embedded therein, and/or associated therewith, and/or attached thereto, a gene therapy vehicle according to Claims 2-14 and 24.

27 A wound healing system according to Claims 26 wherein said matrix material comprises native collagen.

28. A wound healing system according to Claims 26 or 27 wherein said

matrix material comprises collagenous gels or lattices constructed from reconstituted collagen or highly complex mixtures of reconstructed collagen.

29. A wound healing system according to Claims 26-28 wherein said matrix material comprises extra cellular matrix products.

30. A wound healing system according to Claims 26-29 comprising a surgical dressing.

31. A wound healing system according to Claims 26-30 adapted for use in the treatment of acute, and/or chronic, and/or minor, and/or severe, wound healing.

32 A wound healing system according to Claims 26-31 for use in the treatment of cartilage repair, and/or bone repair, and/or muscle repair, and/or connective tissue repair, and/or blood vessel repair.

33. A wound healing system according to Claims 26-32 wherein said system comprises a plurality of cell types from a hair follicle.

34 A wound healing system according to Claim 33 wherein one of said cell types, in addition to said dermal sheath tissue, and/or cells derived therefrom, and/or cells typically closely associated with hair follicles, comprises dermal papilla tissue.

35. A therapeutic composition according to Claims 16-23 wherein said composition comprises a plurality of cell types from a hair follicle.

36. A therapeutic composition according to Claim 34 wherein one of said cell types, in addition to said dermal sheath tissue, and/or cells derived therefrom, and/or cells typically closely associated with hair follicles, comprises dermal papilla tissue.

PATENT COOPERATION TREATY

27/9/99

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MARKGRAAF PATENTS LIMITED
The Crescent
54 Blossom Street
York Y024 1AP
GRANDE BRETAGNE

PCT

NOTIFICATION CONCERNING INFORMAL COMMUNICATIONS WITH THE APPLICANT

(PCT Rule 66.6)

	Date of mailing *(day/month/year)* 24.09.99
Applicant's or agent's file reference FP2400	**REPLY DUE** within 1 month(s) from the above date of mailing
International application no. PCT/GB98/02150	International filing date *(day/month/year)* 17/07/1998
Applicant REYNOLDS-JAHODA, Amanda et al.	

An informal communication took place on 17/09/1999, between the International Preliminary Examining Authority and the applicant / the agent.

Invitation pursuant to Rules 66.2 c), 66.3 and 66.4 of the PCT

Further examination of the international application has revealed that the application fails to meet the requirements of the PCT and the Regulations as explained in the attached note (Form PCT/IPEA/428).

The Applicant is hereby **invited**, within the time limit indicated above, **to submit a written reply** accompanied by amendments.

If no reply is submitted, the international preliminary examination report will reflect the opinion expressed by this Authority.

Name and mailing address of the international preliminary examining authority
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Hebert, W.

Telephone No.+49 89 2399-2152

Form PCT/IPEA/429 (july 1993)

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traité de coopération en matière de brevets

PCT

Application No.: PCT/GB98/02150

Note on an informal communication by telephone with the Applicant

Transmittal of a copy of this note with a time limit of **1 month(s)**

Participants

Applicant: Reynolds-Jahoda A. et al

Agent: Mr. R.C. Docherty

Examiner(s): Seegert, K

Summary of the communication

1. The set of claims filed with the letter of 17 June 1999 had been discussed.

2. The Applicant was informed that the subject-matter of claims 1 - 14 and 16 - 36 met the requirements of the PCT with respect to novelty of inventive step. The use of dermal sheath tissue in gene therapy or any other therapy had not been disclosed in the prior art. Although the prior art disclosed the use of different parts of the hair follicle for the use in gene therapy it would not have been obvious to the skilled person to use the specific part as disclosed in the present application.

2. The novelty objection with respect to claim 15 expressed in the preliminary opinion had not been overcome.

3. The Applicant was further informed that the subject-matter of claims 1 and 2 lacked clarity within the meaning of Article 6 PCT since the claims did not contain every important feature in order to carry out the invention. In particular, the claims did not specify that the dermal sheath tissue/cell had to be genetically modified (see e.g. claim 6).

4. The Applicant clarified that amended figure 1 was for illustration purposes only.

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traîté de coopération en matière de brevets

PCT

Application No.: PCT/GB98/02150

17/09/1999
.....
Date *(day / month / year)*

Seegert, K
.....
Authorized officer of IPEA

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MARKGRAAF PATENTS LIMITED
The Crescent
54 Blossom Street
York Y024 1AP
GRANDE BRETAGNE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	21.04.99
Applicant's or agent's file reference: FP2400	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02150	17/07/1998	18/07/1997

International Patent Classification (IPC) or both national classification and IPC
A61K48/00

Applicant
REYNOLDS-JAHODA, Amanda et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 18/11/1999.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0 Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer / Examiner
Seegert, K

Formalities officer (incl. extension of time limits)
Senkel, H
Telephone No. (+49-89) 2399 8071

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-30 as originally filed

Claims, No.:

1-36 as originally filed

Drawings, sheets:

1/11-11/11 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-26,30-33,35
Inventive step (IS)	Claims	1-36
Industrial applicability (IA)	Claims	

2. Citations and explanations

se separate sheet

Section V

1. Reference is made to the following documents:

   D1: WO-A-9 325 660
   D2: Journal of Investigative Dermatology, vol 105 (1), 1995, pages 14 - 21
   D3: EP-A-0 679 402
   D4: WO-A-8 902 468
   D5: GB-A-2 293 604
   D6: WO-A-9 322 430
   D7: EP-A-0 236 014
   D8: WO-A-9 501 423

2. The subject-matter of the present application basically relates to dermal sheath tissue and/or cells derived therefrom and/or cells closely associated with hair follicles for use in gene therapy.

3. Document D1 (e.g. claims) discloses a method for in vivo gene transfer into keratinocytes or follicular stem cells by using viral vectors or plasmids. The genetically modified cells are used e.g. for the healing of wounds.

   Similarly, document D2 (page 20, left-hand column, last sentence) suggests that keratinocytes derived from hair follicles can be successfully used in gene therapy.

   Document D3 (page 3, lines 9 - 20) discloses a hybrid gel for external use comprising genetically modified skin cells (epidermal or fibroblast cells) containing an expression vector encoding a biologically active substance optionally in conjunction with an antibiotic resistance gene.

   Similarly, documents D4 (claims) and D5 (e.g. example 6) disclose transduced fibroblasts for use in gene therapy, e.g. as a skin graft.

   Document D6 (claims) discloses the use of genetically transformed epidermal

cells for use in e.g. wound treatment.

4. It is noted that the term "dermal sheath tissue and/or cells derived therefrom and/or cells typically closely associated with hair follicles" of claim 1 seems also to cover the particular cell types forming the subject-matter of documents D1 - D6.

   Consequently, in the light of the analysis provided above the subject-matter of claims 1 - 26, 30 - 33 and 35 seems to lack novelty under Article 33 (2) PCT over the documents D1 and D3 - D6.

   As for claim 15 it is further noted that the vector according to the present application seems to be commercially available (see page 22 of the description). Consequently, claim 15 is deprived of novelty. Vectors covered by the scope of claim 15 seem further to be disclosed in e.g. document D3 - D6.

5. Claims 27 - 29 and 34, 36 appear to be novel since their specific technical features have not been disclosed. However these additional features seem to be a normal design procedure to the skilled person thus not involving an inventive step under Article 33 (3) PCT.

6. For completeness' sake reference is further made to documents D7 (claims) and D8 (claims) which disclose the use of cultured cells derived from hair follicles for the improvement of hair growth. Since the subject-matter as claimed in independent claims 1, 2, 16 and 26 is not restricted to a genetically modified material the teaching of D7 and D8 would also be considered novelty destroying to the present application.

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:
MARKGRAAF PATENTS LIMITED
The Crescent
54 Blossom Street
York YO24 1AP
UNITED KINGDOM

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference FP2400	Date of mailing *(day/month/year)* 01/12/1998 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/GB 98/ 02150	International filing date *(day/month/year)* 17/07/1998
Applicant REYNOLDS-JAHODA, Amanda et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Heike Zoglauer

Form PCT/ISA/220 (January 1994)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the internati nal application i French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
FP2400		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 02150	17/07/1998	18/07/1997
Applicant		
REYNOLDS-JAHODA, Amanda et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant
   - [X] the text has been established by this Authority to read as follows:

   Gene therapy vehicle comprising dermal sheath tissue

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02150

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K48/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 25660 A (BRIGHAM & WOMENS HOSPITAL) 23 December 1993 see claims	1-36
X	EP 0 679 402 A (JAPAN RES DEV CORP ;YOSHIDA SUSUMU (JP); CUTHBERTSON R ANDREW (US)) 2 November 1995 see page 3, line 9 - line 20	1-36
X	WO 89 02468 A (WHITEHEAD BIOMEDICAL INST ;HOWARD HUGUES MEDICAL INST (US)) 23 March 1989 see claims	1-36
X	GB 2 293 604 A (BRITISH TECH GROUP) 3 April 1996 see example 6	1-36

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 1998	01/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 22430 A (BAYLOR COLLEGE MEDICINE ;UNITED SATES OF AMERICA (US)) 11 November 1993 see claims	1-36
X	EP 0 236 014 A (UNIV DUNDEE) 9 September 1987 see claims	1-5, 11-14, 16,19, 21,22, 25,26, 31-36
X	WO 95 01423 A (UNIV PENNSYLVANIA ;UNIV NEW YORK (US)) 12 January 1995 see claims	1,2,5,8, 11-14, 16,21, 22,25, 26,31,32
A	MOLL I.: "Proliferative Potential of Different Keratinocytes of Plucked Human Hair Follicles" JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 105, no. 1, 1995, pages 14-21, XP002084482	1-36

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/02150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9325660 A	23-12-1993	US 5423778 A	13-06-1995
		AU 4535693 A	04-01-1994
		EP 0644929 A	29-03-1995
		JP 8509356 T	08-10-1996
		US 5661132 A	26-08-1997
		US 5697901 A	16-12-1997
EP 0679402 A	02-11-1995	AU 1766595 A	02-11-1995
		CA 2147810 A	26-10-1995
		JP 8196271 A	06-08-1996
WO 8902468 A	23-03-1989	AT 117375 T	15-02-1995
		DE 3852823 D	02-03-1995
		DE 3852823 T	24-05-1995
		EP 0378576 A	25-07-1990
		EP 0633318 A	11-01-1995
		JP 3500124 T	17-01-1991
		US 5460959 A	24-10-1995
GB 2293604 A	03-04-1996	AU 694957 B	06-08-1998
		AU 3481695 A	09-04-1996
		CA 2198379 A	28-03-1996
		EP 0783568 A	16-07-1997
		WO 9609373 A	28-03-1996
		JP 10505756 T	09-06-1998
WO 9322430 A	11-11-1993	AU 4220793 A	29-11-1993
		CA 2134675 A	11-11-1993
		EP 0652948 A	17-05-1995
		JP 8503844 T	30-04-1996
EP 0236014 A	09-09-1987	AU 598235 B	21-06-1990
		AU 6915187 A	27-08-1987
		CA 1306416 A	18-08-1992
		DE 3771747 A	05-09-1991
		GR 3002975 T	25-01-1993
		JP 62246508 A	27-10-1987
		US 4919664 A	24-04-1990
WO 9501423 A	12-01-1995	US 5556783 A	17-09-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/02150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9501423 A		AU 7213294 A	24-01-1995
		US 5756094 A	26-05-1998

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HARRISON, Michael
Harrison Goddard Foote
Belmont House
20 Wood Lane
Leeds LS6 2AE
ROYAUME-UNI

24.NOV.1999*021110

Date of mailing (day/month/year) 16 November 1999 (16.11.99)	
Applicant's or agent's file reference FP2400 FP8038WO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB98/02150	**International filing date** (day/month/year) 17 July 1998 (17.07.98)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
MARKGRAF PATENTS LIMITED The Crescent 54 Blossom Street York YO24 1AP United Kingdom		
	Telephone No. 01904-610586	
	Facsimile No. 01904-610909	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
HARRISON, Michael Harrison Goddard Foote Belmont House 20 Wood Lane Leeds LS6 2AE United Kingdom		
	Telephone No. +44 113 225 8350	
	Facsimile No. +44 113 230 4702	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☒ the International Preliminary Examining Authority
☐ the designated Offices concerned
☒ the elected Offices concerned
☒ other: MARKGRAAF PATENTS LIMITED

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer N. Lindner
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

002954964

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* **FP2400**

Box No. I TITLE OF INVENTION

GENE THERAPY VEHICLE

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

REYNOLDS-JAHODA, AMANDA
26 Maison Dieu
RICHMOND
North Yorkshire
DL10 7AU
UNITED KINGDOM

[X] This person is also inventor.

Telephone No. 01748 824856

Facsimile No. 01748 824856

Teleprinter No.

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

This person is applicant for the purposes of: [X] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

JAHODA, COLIN ALBERT BUCHANAN
26 Maison Dieu
RICHMOND
North Yorkshire
DL10 7AU
UNITED KINGDOM

This person is:

[ ] applicant only

[X] applicant and inventor

[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

This person is applicant for the purposes of: [X] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MARKGRAAF PATENTS LIMITED
THE CRESCENT
54 BLOSSOM STREET
YORK YO24 1AP
UNITED KINGDOM

Telephone No. 01904 610586

Facsimile No. 01904 610909

Teleprinter No.

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ AP **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* . . . . . .

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ AL Albania . . . . . .
- ☒ AM Armenia . . . . . .
- ☒ AT Austria . . . . . .
- ☒ AU Australia . . . . . .
- ☒ AZ Azerbaijan
- ☒ BA Bosnia and Herzegovina . . . . . .
- ☒ BB Barbados
- ☒ BG Bulgaria . . . . . .
- ☒ BR Brazil . . . . . .
- ☒ BY Belarus . . . . . .
- ☒ CA Canada
- ☒ CH and LI Switzerland and Liechtenstein
- ☒ CN China . . . . . .
- ☒ CU Cuba . . . . . .
- ☒ CZ Czech Republic . . . . . .
- ☒ DE Germany . . . . . .
- ☒ DK Denmark . . . . . .
- ☒ EE Estonia . . . . . .
- ☒ ES Spain . . . . . .
- ☒ FI Finland . . . . . .
- ☒ GB United Kingdom
- ☒ GE Georgia . . . . . .
- ☒ GH Ghana . . . . . .
- ☒ GM Gambia
- ☒ GW Guinea-Bissau . . . . . .
- ☒ HR Croatia
- ☒ HU Hungary . . . . . .
- ☒ ID Indonesia
- ☒ IL Israel . . . . . .
- ☒ IS Iceland
- ☒ JP Japan . . . . . .
- ☒ KE Kenya . . . . . .
- ☒ KG Kyrgyzstan . . . . . .
- ☒ KP Democratic People's Republic of Korea . . . . . .
- ☒ KR Republic of Korea . . . . . .
- ☒ KZ Kazakhstan . . . . . .
- ☒ LC Saint Lucia
- ☒ LK Sri Lanka
- ☒ LR Liberia
- ☒ LS Lesotho . . . . . .
- ☒ LT Lithuania
- ☒ LU Luxembourg
- ☒ LV Latvia
- ☒ MD Republic of Moldova . . . . . .
- ☒ MG Madagascar . . . . . .
- ☒ MK The former Yugoslav Republic of Macedonia . . . . . .
- ☒ MN Mongolia
- ☒ MW Malawi . . . . . .
- ☒ MX Mexico . . . . . .
- ☒ NO Norway
- ☒ NZ New Zealand . . . . . .
- ☒ PL Poland . . . . . .
- ☒ PT Portugal . . . . . .
- ☒ RO Romania
- ☒ RU Russian Federation . . . . . .
- ☒ SD Sudan
- ☒ SE Sweden
- ☒ SG Singapore
- ☒ SI Slovenia . . . . . .
- ☒ SK Slovakia . . . . . .
- ☒ SL Sierra Leone
- ☒ TJ Tajikistan . . . . . .
- ☒ TM Turkmenistan . . . . . .
- ☒ TR Turkey . . . . . .
- ☒ TT Trinidad and Tobago . . . . . .
- ☒ UA Ukraine . . . . . .
- ☒ UG Uganda . . . . . .
- ☒ US United States of America . . . . . .
- ☒ UZ Uzbekistan . . . . . .
- ☒ VN Viet Nam . . . . . .
- ☒ YU Yugoslavia . . . . . .
- ☒ ZW Zimbabwe . . . . . .

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ . . . . . .
- ☐ . . . . . .

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 18 July 1997	9715085.8	GB		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s):

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*: Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	3
description (excluding sequence listing part)	:	30
claims	:	5 6
abstract	:	1
drawings	:	11
sequence listing part of description	:	-
Total number of sheets	:	50

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: 23/77

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Markgraaf Patents Ltd

MARKGRAAF PATENTS LIMITED

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

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Date of receipt of the record copy by the International Bureau: